STAIN TECHNOLOGY

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A PRACTICAL STAINING RACK

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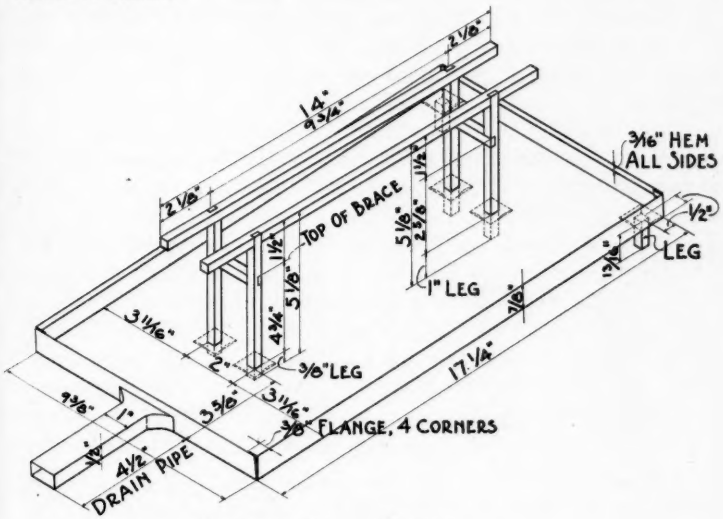
The staining rack shown in the accompanying sketch was devised by the writer and has been in use for several years without being considered worthy of public description. Frequent recent comments about it, however, have finally convinced the writer that it may be useful to others.



STAINING RACK

The sketch is very largely self-explanatory; but a few additional matters of detail are needed, e.g.:

1. The staining rack is of $\frac{5}{16}$" brass bars.
2. All bar joints are to be notched, lapped and brazed.
3. Four of the six legs are to be continuations of the vertical bar-struts of the rack.

4. All the legs shall be secured to the basin with built-up solder reinforcing around legs on underside of basin.
5. Basin to be of 16 oz. cold rolled sheet copper.
6. All basin corners to be brazed.
7. Drain pipe to be seamless copper tube.
8. Pipe to be brazed to basin.
9. Drain pipe to have same slope as basin, e.g., about ¾″ to the running foot.

The advantages of such a rack are rather self-evident, but may be emphasized as follows: One may stain 12 slides at a time without any difficulty. Rubber tubing connected to a faucet is used for washing. The slant of the staining basin offers a quick outflow of stain and water.

Since the above note was written, a staining rack embracing all the features here described has become available commercially and is announced in the advertising pages of this issue.

A TECHNIC FOR THE SECTIONING OF MAMMALIAN OVA AND BLASTOCYSTS[1]

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ABSTRACT.—**Ova and blastocysts are fixed in Bouin's fluid. Gradual dehydration thru the alcohols to 70% follows. Eggs and blastocysts are stored in 70% alcohol.**

The egg.—The 70% alcohol is reduced gradually to 30%. Agar (1.7 g.) is dispersed in 98 cc. distilled H_2O by heat. Hot agar (5–6 drops) is placed on a 50 x 75 mm. microscope slide. After the agar has cooled slightly, the egg is placed in the agar and the agar allowed to gel. The agar is trimmed to about a 2–3 mm. block. The agar with the egg is pushed on a piece of lens paper which is folded about it. The folded lens paper with agar is placed in Bouin's fluid, and the following day run up to 70% alcohol. It is then run back to water and stained with Heidenhain's iron hematoxylin. After being passed thru the alcohols, it is cleared in cedar oil. Embedding in paraffin follows.

The blastocyst.—The blastocyst is stained with Delafield's hematoxylin. It is carried to absolute alcohol. The alcohol is reduced to a minimum and the bottom of the dish coated with glycerin. Cedar oil is substituted for the alcohol. The cedar oil is washed out with a 2–3 minute change of xylol which is replaced with paraffin-xylol. Three changes of paraffin-xylol are made during the next 2–3 hours. Five changes of paraffin are made during 1½ hours of infiltration. All changes of fluid are made under the microscope and by drawing the fluid off and adding new fluid with pipets rather than moving the blastocyst. Between changes of paraffin the bulbs are removed and the pipets placed in a warm oven.

The small size of the mammalian ovum and blastocyst has necessitated the development of special technics for their embedding and sectioning. Hill (1910) and Hill and Tribe (1924) working with the cat fastened the egg to a piece of tissue, brain cortex or fetal membrane, by means of a dilute solution of photoxylin (1–2%). A rather

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elaborate but effective method of handling and infiltrating eggs has been described by Heuser and Streeter (1929) and also by Gregory (1930). By this method ova are transferred from dish to dish by means of fine pipets. At the time of infiltration electrically heated pipets and plates are used. A complete set of equipment as described by Gregory was made and used satisfactorily in this laboratory.

Mammalian ova and blastocysts are fragile and every time either is handled there is a chance of injury. If, however, the egg can be placed in some suitable mechanical carrier which will not damage it, the egg may be moved as quickly and easily as larger pieces of tissue. The finding of a suitable carrier for the egg was reduced to the finding of a gel which could be kept in a sol form at a low enough temperature to permit its use without causing heat injury to the egg and still be of practical use. Agar as used for bacteriological work was found to be satisfactory. It is easy to handle; it remains liquid at a comparatively low temperature; and it does not injure during subsequent treatments.

All the ova and blastocysts recovered for this study were fixed in Bouin's fluid and dehydrated gradually thru the series of alcohols to 70%, remaining in each 30 to 45 minutes. In changing the alcohols, one-third of the original fluid was drawn off and substituted by a larger volume of the next alcohol. Then two-thirds of the alcohol was removed and replaced with fresh alcohol; this was repeated several times. The standard embryological dish was used and the changes were made under a low power microscope.

The Egg

The 70% alcohol in which the egg has been stored is reduced gradually to a 30% solution. Clean pure agar (1.7 g.) is added to 98 cc. of distilled water and dispersed by heating almost to the boiling point in a covered beaker. The egg is drawn into a fine pipet. (This is the only time the egg has to be moved.) A 50 x 75 mm. microscope slide is placed under the microscope, and 5–6 drops of hot agar placed on the slide. When the agar has cooled sufficiently to prevent heat injury to the egg, the egg is placed in the agar, and the agar permitted to gel. A little distilled water is dropped on the agar; this acts only as a lubricant, and by means of a sharp blade the excess agar is cut from the egg. Each step is carried out under a low power microscope. The remaining agar, about 2–3 mm. square, with the egg in the center is pushed gently from the wet slide onto a piece of lens paper which is then folded about the agar as described

by Guyer (1930). The folded lens paper is placed in Bouin's fluid and the following day run up to 70% alcohol.

Two-celled eggs and occasionally the more advanced stages require orientation. In this case a larger amount of agar is placed on the slide; when it is cool the egg is added and, by means of a hair fixed in a hemostat, the egg may be oriented. If the egg is not oriented properly in the agar, it may be reoriented without harm. As much agar as possible is trimmed from the egg, and the egg with the remaining agar is re-embedded in another agar sol. After the agar has congealed sufficiently, it is cut into an oblong block 3 x 10 mm., the egg being a short distance from one end. The long side of the block should be parallel to the division line between the cells. The end not containing the egg may be cut any shape to differentiate the ends. The agar is later oriented in the paraffin block.

The agar wrapped in the lens paper is transferred easily from dish to dish with the aid of small forceps. The very gradual changes of alcohols as suggested previously for the unprotected egg are unnecessary, because the agar retards contact of the new solution with the egg altho it is very efficient for the exchange of the liquids. (For a discussion of gel structure and formation see Gortner, 1929.)

The agar in the lens paper is run from the 70% alcohol back to water from which it is placed in ferric alum for four hours. It is then stained overnight in Heidenhain's iron hematoxylin. The agar is then passed thru the alcohols (30, 50, 70, 80, 95%, and absolute) leaving it 45 minutes in each. At this time the agar is black. The agar is "cleared" in cedar oil, and allowed to remain in cedar oil at least one hour after it sinks (overnight is a convenient time for this). It is washed with xylol 2–3 minutes and then placed in paraffin-xylol for three hours. Infiltration is accomplished by changes of paraffin (56° C.) every 15 minutes for 1–1½ hours or until the cedar oil is all removed. The warm lens paper is now unfolded. The small block of agar, which is observed easily, is transferred into the embedding paraffin (56° C.) by means of a warm forceps.

No marker is necessary because the black color of the agar indicates clearly its position in the block. The egg is then sectioned at 3–4 μ and placed on slides. At this point the previous staining and the agar are of much benefit; the agar and egg are observed with ease in the paraffin ribbon. A few wrinkles may appear in the agar, but they are removed readily by careful manipulation of very fine dissecting needles, the operation being watched thru the microscope. By this means only the slides containing the sectioned egg need be saved and no time is lost hunting for the egg. After the slide has dried it

is put thru xylol, the alcohols, and water, at which point it is destained carefully with ferric alum. The usual methods of technic are followed from this point to cover-slipping. A counterstain may be given if desired.

The egg stands out clearly and distinctly in the agar which is destained completely.

Fig. 1. A rat ovum, recovered from the follicle, sectioned by the method described herein. × 740.

The Blastocyst

For obvious reasons agar could not be used for the blastocysts; they are very fragile and will fragment upon the slightest provocation. Transferring thru the alcohols and other fluids is far more hazardous than the same operation with eggs. A method to reduce handling to a minimum has been developed. Only one transfer is necessary and that cannot be eliminated.

After the blastocyst is found in the flushing fluid, it is transferred to a clean embryological dish containing Bouin's fluid. It is allowed to fix for 12–24 hours after which it is dehydrated gradually to 70% alcohol. It is then stained with Delafield's hematoxylin diluted to one-third strength with distilled water. Following Gregory's suggestion, a very small amount of HCl should be added to the stain. This temporary stain is desirable for locating the blastocyst in the paraffin block. The blastocyst is then carried to absolute alcohol,

the changes from 70% differing by 5%. At this time the bulk of the alcohol is removed and by gentle rotation the inside of the dish is coated with glycerin. Cedar oil is then substituted for the alcohol and the blastocyst is left in the cedar oil until one hour after it sinks (overnight is convenient). A precaution to be observed at this point is that the dish should be filled only one-half to two-thirds with cedar oil. A full dish seems to inhibit the normal "sinking" of the material.

The cedar oil is washed out with a 2–3 minute change of xylol and replaced with paraffin-xylol for 2–3 hours. Three changes of paraffin-xylol (24 g. per 100 cc.) are made during this time. Five changes of paraffin (56° C.) are made during the 1½ hours of infiltration. The bulbs are removed from the pipets and the pipets placed in the warm oven between changes. The microscope is placed near the oven so that the changes can be made quickly and effectively.

At the time of embedding the blastocyst, the embryological dish is placed inside a Petri dish containing ice-water. By means of a hair mounted in a hemostat the blastocyst is raised gently until a film of solid paraffin has formed over the bottom of the dish. The blastocyst is allowed to rest on the paraffin and oriented into position; when the paraffin has hardened, the block may be marked with a needle to indicate the position of the blastocyst. A marker in the paraffin is not necessary because of the temporary staining of the blastocyst.

The blastocyst is then sectioned as described for the egg. The ribbon is observed under the microscope to guard against wrinkles in the tissue. The sections are destained and then stained permanently with Heidenhain's iron hematoxylin.

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A HISTOLOGICAL STAIN FROM THE BLACK WALNUT (JUGULANS NIGRA L.)

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The aim of this work was to investigate the dye found in the hulls of the black walnut (*Jugulans nigra*) and to determine its value as a histological stain.

So far as the authors have been able to ascertain there have been no previous investigations in this field altho references have been found which indicate that the black walnut might be a possible source of dye material. During the early history of this country homespun goods were often dyed with the extract of walnut hulls. The extract was obtained by boiling some of the blackened hulls in water. When goods were boiled in this extract they took on a good brown color which did not fade or run. Altho this seems to have been quite common practice in this country and Europe, apparently there has been no investigation made as to the possibilities of this dye material as a histological stain.

It was the opinion of the authors that if certain tests were made upon the properties of the walnut dye before attempting to do any actual staining, they would greatly facilitate the work which followed. For example, in order to prepare staining solutions and also to obtain a pure dye, the action of various solvents was studied. In experimenting with various mordants much time was saved by testing the ability of the dye to react with various chemicals before actually trying them as mordants. It was also the intention of the authors to attempt to determine the structure of the dye, but on account of the difficulties encountered and the time available it has been necessary to defer such investigation until a later date.

The method of testing the solubility of the dye consisted in extracting 0.5 g. portions of the ground, dried walnut hulls with 100 cc. portions of various organic and inorganic solvents. The extraction process was continued for 24 hours and was accompanied by occasional agitation of the liquid. Distilled water was found to be the best solvent for extraction of the dye material. Two parts of distilled water to one part of the dried, powdered hulls were used. Extraction was carried on for 24 hours and the resulting liquid was filtered. When this liquid was evaporated to dryness, over a steam bath, the residue was a hard, black, shiny, crystal-like substance with

a caramel-like odor. A stock solution of the dye was then prepared by saturating distilled water with the dye residue. It was found necessary to add a few thymol crystals to this stock solution to preserve it.

Experimental procedure, using standard methods of staining on the slide, showed that good results could not be obtained by using the stock solution or any of its dilutions. It was found that a mordant was necessary to have the stain "take". Of the various mordants tried, ferric alum was found to produce the best results.

The use of aqueous solutions of the dye extract following the mordanting of the sections for 1 hour in a 5% solution of ferric alum, shows the following results:

The stain is a nuclear stain producing a brown coloration in the chromatin material.

The stain is slow in action. Best results were obtained by the use of the stain for 24 hours.

Some tissues were not overstained by being left in the staining solution for 7 days.

Best results were obtained following the use of picro-formol, picro-sulfuric, and mercuric chloride fixatives.

THE VAPOR METHOD OF CHANGING REAGENTS AND OF DEHYDRATION

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In making permanent preparations from aceto-carmine smears the distortion and shrinkage formerly met with have been largely eliminated by a differential vapor pressure method, briefly described below. This method could have valuable extensions to other materials and reagents and to other fields.

The slide bearing the prepared smear should have a minimum of liquid, with the cover glass chosen as small as feasible, e. g., 15, 18, 22 mm. squares for single pairs of salivary glands and 22 x 40 mm. (18 x 40 mm. if obtainable) oblongs for four or more pairs to the slide. This preparation is put directly, without sealing the edges, into a tightly covered jar kept saturated with vapor of 95% alcohol. The jar should be at least 4 inches deep, preferably 6, and of bottom area proportional to the number of slides to be handled at once. The walls and the cover (of large jars) are lined with wick material—blotting paper or layers of paper toweling or coarse filter-paper, and the bottom is covered more thickly than the sides. This material is kept soaked with 95% alcohol with a slight excess of free liquid. The slides are stood on end around the walls with their lower ends on the very wet paper on the bottom. When the jar is covered tightly the interior soon becomes saturated with alcohol vapor. The alcohol slowly condenses on the surfaces of the slide, mingles with the aceto-carmine at the edges of the cover and seeps under. Displacement occurs progressively thru a slight drainage at the bottom of the slide and thru evaporation into the acetic-free atmosphere which is relatively dry with respect to water. Absorbers for the materials to be removed by evaporation could be used to hasten the process (e. g., CaO for H_2O and acetic acid).

Twenty-four hours is usually sufficient for the dehydration, but no harm follows from leaving the slides several days, providing the excess of free liquid is maintained.

After the vapor treatment the slides are immersed in liquid 95% alcohol in Coplin jars or staining trays and left some hours or days.

The covers are then removed by laying each slide on its back in a bath of 95% alcohol in a shallow Petri dish, and slipping under one corner or edge of the cover glass a thin wedge-shaped needle tip.

Meanwhile the opposite corners of the cover glass should be firmly held in place with the fingertips to prevent the least slippage. When the cover glass has been lifted free and the space below has flooded with alcohol, the cover is removed and cleaned of any adhering material.

Practically all of the material adheres to the slide if the slide has been pretreated with a very thin dried film of albumen as advised by Dr. Hans Bauer.[1] The albumen solution is made by mixing together 100 cc. distilled water and 25 g. powdered egg albumen (Merck). After the excess of albumen has settled out, the supernatant liquid is decanted, diluted to 200 cc. and preserved by 1 g. thymol. A drop of the solution is spread evenly and thinly over the entire slide by scraping with the end of a second slide whose edge is smooth and unchipped.

The slide is lifted from the bath, drained and laid on its back. The material is covered by two or three drops of thinned euparal and the cover is replaced, avoiding bubbles. This stage should be carried thru rapidly in a dry atmosphere without breathing on the mount. The excess of thin euparal is squeezed out between the folds of a paper towel.

No other reagents beyond 95% alcohol and euparal are needed for perfect results, absolute alcohol and xylol being eliminated from the process, but other dehydrants such as dioxan or suitable higher alcohols can be used instead of the ethyl alcohol. "Diaphane" (which can be obtained from Will Corporation, Rochester, N. Y.) can be used instead of euparal in mounting directly from 95% alcohol and is probably better.

[1]Bauer, Hans. Notes on permanent preparations of salivary gland chromosomes. Drosophila Information Service, **6**, 35–6. 1936. Carnegie Institution of Wash., Department of Genetics, Cold Spring Harbor, N. Y.

PERMANENT POLLEN TUBE SLIDES WITH THE VAPOR METHOD OF CHANGING REAGENTS AND DEHYDRATION

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Pollen tube studies from ovaries of *Reseda* species (mignonette), in the family Resedaceae, mounted in a temporary medium such as lactic acid, can be made permanent mounts in balsam with little change in the tissues due to shrinkage or other distortions by the use of the vapor method of changing reagents and dehydration.[1] The use of dioxan[2] in the vapor jar fixes the pollen tubes embedded in ovary tissue and replaces the lactic acid with dioxan, and prepares the material for permanent mounting in balsam.

The procedure for preparation of pollen tube slides was devised by Buchholz[3] and is used for pollen tube studies on *Datura* at the present time. Modifications in technic were made for *Reseda* species, because these ovaries lack a style or stylar tissue in which pollen tubes are generally found. In Resedaceae the pollen tubes grow along the ovary wall, thru parietal placenta tissue. This morphological feature made it necessary to prepare permanent mounts, since the pollen tubes are easily destroyed and moved out of place when mounted in a temporary medium such as lactic acid.

Pollinated flowers are taken into the laboratory where the ovaries are removed and placed in water heated to about 75° C., and kept there for 20 or 30 seconds. The ovary is then removed to killing fluid of formalin and alcohol (6% formalin, 50% alcohol) where it remains for about an hour. After fixation in the killing fluid, each ovary is transferred to water for washing and dissection. The ovaries are dissected under a microscope into the respective carpel parts composing the ovary which is generally three in the case of *Reseda* species. The carpel possesses a parietal placenta with numerous ovules attached to it, and a stigmatic surface upon which the pollen collects and germinates.

Following fixation, dissection and washing, the carpel was placed

[1]Bridges, Calvin B. 1937. The vapor method of changing reagents and of dehydration. Stain Techn., **12**, 51.

[2]Dioxan, chemical solvent secured from Carbide and Carbon Chemicals Corp., 30 East 42 Street, New York, N. Y.

[3]Buchholz, J. T. 1931. The dissection, staining and mounting of styles in the study of pollen-tube distribution. Stain Techn., **6**, 13–24.

in stain. A stain used by Buchholz composed of 80 cc. of a 1% aqueous solution of acid fuchsin and 20 cc. of a 1% aqueous solution of light green SF yellowish was employed in this work. The material was kept in stain for about 10 minutes, washed in water and transferred to lactic acid.

Lactic acid softens the tissue and clears it. This process of clearing and softening takes about 12 hours, after which the entire carpel is transferred to a clean slide with excess lactic acid added to insure proper mounting with the cover slip. A No. 1 or No. 2 cover slip is placed on the material which is spread out on the slide by application of pressure on the cover slip with a needle. This flattening process is done under a microscope to determine the amount of pressure necessary to show the pollen tubes which are embedded in ovary tissue. When good views of pollen tubes are available the slide is ready for permanent mounting.

The slide containing the pollen-tube material, which is mounted in lactic acid, is placed in a dioxan vapor jar. The dioxan vapor penetrates between the slide and cover slip, fixes and hardens the pollen tubes and ovary tissue, and replaces the lactic acid with dioxan. This process of reagent change and dehydration preserves the mounted objects in almost the exact position secured after pressing out the material in lactic acid. There is practically no distortion or shrinkage in the material due to the change of reagents.

Slides are kept in the vapor jar for about 8 hours and then transferred to a jar filled with dioxan liquid. The slide is kept in the liquid dioxan for about 5 or 6 hours. One should not leave the slide in dioxan for too long a period on account of slight destaining which is not desired if the slide is not originally over stained.

The cover slip often comes off in the liquid. Removal of the cover slip by use of a needle, however, does not injure the tissue on the slide. The slide is transferred to a Petri dish where the cover slip may be removed and then mounted in balsam or euparal.

The use of a very thin dry layer of egg albumen on the slide before preparation of the mount, as suggested by Dr. Hans Bauer and described by Bridges, (see footnote 1), will hold all material to the slide.

Permanent pollen-tube mounts have been kept for several months with no apparent change in the tissues. Slides may be filed away and kept for future reference just as any permanent mounted specimen.

IMPROVED SERIAL SECTIONING OF YOLK-RICH MATERIALS

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The preparation of thin serial sections of yolk-rich materials, such as the eggs of certain vertebrates and invertebrates, is notoriously precarious. The heavier amphibian zygotes are rarely cut less than 10 μ thick; even then the yolk-rich portions are usually badly distorted or lost. The rubber-asphalt-paraffin method is of some aid, but by no means overcomes the difficulty.

The writer has found it necessary to prepare serial sections, varying from 4–25 μ, of several hundred zygotes and other early stages of the Pacific coast newt, *Triturus torosus*. It seems quite probable that many others have discovered the method described below. It was never communicated to me, however, altho the problem has been continually discussed with persons interested in microtechnic, nor has it been encountered in the literature. Apparently, then, it is not generally known.

The zygote of the Pacific coast newt is about 2.5 mm. in diameter, seen from the top. It is extremely rich in yolk and presents the difficulties usual to such materials. I have experimented with (1) the addition of rubber and beeswax to the paraffin, (2) paraffins of varying degrees of hardness, (3) mixtures of soft and hard paraffins, (4) short and prolonged treatment in the alcohols, xylol, and paraffin. Only by alteration of a purely mechanical detail, however, have satisfactory thin serial sections been obtained consistently.

To cut sections 4 or 5 μ thick I use 60–68° paraffin; for 8 to 12 μ a 55–57° paraffin is desirable. Dehydrate thoroly, pass thru xylol, xylol-paraffin, paraffin, and embed in the usual manner. Success in sectioning the material depends upon the manner in which the microtome wheel is operated. *The customary uniform rate of rotation, whether fast, medium or slow, is the source of difficulties.* The secret lies in two points: (1) The microtome wheel is rotated rapidly thru almost a complete cycle, and stopped abruptly just as the paraffin block strikes the knife. The sudden contact produces a very firm adhesion of successive sections. (2) The remainder of the cycle is accomplished very slowly; during this time the knife is passing thru the object. This abrupt change of rate is easily accomplished: the rapid part of the cycle is terminated by allowing the wrist or hand to come in contact with the table. A slight bounce is inevitable but this

does no harm; the firm attachment of the sections has been accomplished and the object itself has not yet been reached. The very slow rate at which the knife passes thru the object hardly compresses the paraffin block at all, and reduces to a minimum all distortion of form and displacement of yolk granules. This whole process is mastered within a few minutes and takes much less time than one might imagine, particularly since the major portion of the cycle is soon performed with extreme rapidity. The only rival of this method that I know is to paint the face of the paraffin block with celloidin as each section is cut, a process far more tedious.

Unless particular circumstances dictate otherwise, *the portion of the material poorest in yolk content* (e. g., the upper dark hemisphere of amphibian eggs) *should strike the knife first.*

RAPID EMBEDDING WITH HOT LOW-VISCOSITY NITROCELLULOSE

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ABSTRACT.—**A rapid method for embedding with low-viscosity nitrocellulose is described. Advantage is taken of the greater penetrating power of low-viscosity nitrocellulose than that of common varieties of celloidin. Fixation, dehydration and infiltration are carried out in screw-topped jars in the incubator at 56° C. The whole procedure from fixing to sectioning can be finished in 15–30 hours, and sections as thin as 6 to 10 μ may be cut without difficulty.**

For about ten years celloidin sections have been used extensively in this laboratory for teaching purposes; in the hands of beginners the celloidin method proved easier to handle, and yielded preparations superior to those prepared by the paraffin method. But a serious objection to the celloidin technic was the days or even weeks required; this frequently determined the use of the paraffin method. Wetmore (1932) and later Walls (1932) described a more efficient celloidin technic based on Jeffrey's original method; Walls gave an excellent description of the hot celloidin technic employed in his laboratory. It was found possible to use a temperature of 45–60° C. during the infiltration and thickening processes without injury to animal tissues; the pressure produced by evaporation of the solvent facilitated the penetration of hot celloidin into the tissue. It was also found that the number of concentrations of celloidin used in the process of infiltration might be limited to three or five. Ruby (1933) introduced into this laboratory the use of low-viscosity nitrocellulose as an embedding medium; since then our attention has been directed toward simplifying the technic, especially toward reducing the time required. Davenport (1934), working with low-viscosity nitrocellulose, noticed that even "22% solution of the low-viscosity material was sufficiently fluid to penetrate after direct transfer of tissue from alcohol-ether to it without using intermediate concentrations." Results in this laboratory agree with Davenport's observation, and indicate that low-viscosity nitrocellulose penetrates better than celloidin. The attempt further to reduce the time required for infiltration by using low-viscosity nitrocellulose at high temperature has proved so successful that this method has almost supplanted the paraffin technic in this laboratory.

Method

A. Fixation. Fix pieces of tissue, not thicker than 2–3 mm., for an hour at 56° C., or 2–5 hours at 37° C., in 10% neutral formol, Bouin, Susa or Carnoy II. (Screw-topped jars were used when the tissue was treated in the incubator during any step of fixation, dehydration or infiltration.)

B. Washing. Wash in several changes of distilled water, one hour each at room temperature. In case of fixation in Carnoy II, treat the tissue for 2 hours at 56° C., with 3–5 changes of absolute ethyl alcohol to remove the chloroform.

C. Dehydration. This should be carried out at 56° C., as follows:

1. 70% ethyl alcohol, 2 changes half an hour each (iodized alcohol for half an hour if tissue was fixed in fluid containing mercuric chloride).
2. 80% ethyl alcohol, 2 changes half an hour each.
3. 95% " " " " " " " "
4. Absolute ethyl alcohol, 2 changes half an hour each.
5. Absolute alcohol and ether mixture, one hour.

D. Infiltration with Nitrocellulose.[1] Make solutions of nitrocellulose in 3 different concentrations as follows:

For the 10% solution, 10 g. of nitrocellulose (already moistened with alcohol for shipping purposes) is treated with 50 cc. of absolute ethyl alcohol, and then 50 cc. of ether is added.

For the 25% solution, 25 g. of nitrocellulose, 45 cc. of alcohol and 55 cc. of ether are used.

For the 50% solution, 50 g. of nitrocellulose, 40 cc. of alcohol and 60 cc. of ether are used.

It is advisable to make several liters of each concentration, and to allow ample time for impurities to settle.

Infiltrate at 56° C., as follows:

1. 10% solution, one hour.
2. 25% solution, overnight.
3. 50% solution, 2–3 hours.

E. Embedding and Hardening. Transfer pieces of tissue directly to a fiber block moistened with alcohol-ether. Place enough 50% nitrocellulose around the tissue to ensure good embedding. Then immerse the blocks for about one hour in 2 changes of chloroform for

[1]Nitrocellulose R. S. ½ second, viscosity 3/20–4/20 (A product of the Hercules Powder Company, Parlin, N. J., was used.) The authors wish to thank the Hercules Powder Company for their generous donation of nitrocellulose during the early stage of the development of this method.

hardening, and finally carry them through 3 changes of 80% ethyl alcohol within one hour.

F. Cutting. Section blocks of tissue while wet with 80% alcohol.

In some cases it was found necessary to introduce a slight modification of the technic, especially when sections of 25–100 μ were desired. To make thick sections more pliable during the cutting and subsequent handling, the 50% solution of nitrocellulose was omitted, and the final infiltration and embedding was done in 25% nitrocellulose. It was sometimes helpful to wrap a strip of gummed paper around the fiber block, letting the paper protrude sufficiently to retain the necessary amount of nitrocellulose. With some tissues, such as liver, adrenal, pancreas or spleen, the infiltration time could be reduced to 3 or 4 hours.

Results

The hot low-viscosity nitrocellulose method described herein requires much less time than the standard celloidin method; indeed, it is only slightly slower than the paraffin technic. The whole procedure of fixation, dehydration, infiltration and embedding can be accomplished within 30 hours. When only thick sections are desired, it is possible to reduce the time still further. In many instances animal material obtained and fixed in the morning was sectioned late in the evening of the same day. Neither distortion of tissues nor perversion of staining properties resulted from the speed of the procedure or the high temperature used. The method was tried on a considerable variety of human and animal tissues, and was found applicable likewise to pathological material. A considerable number of such tissues, including a large series of testicular tumors, gave invariably good results. The rapidity of the method should make it practicable for the pathologist, and in students' work, which usually calls for a limited number of sections. No difficulty was encountered in cutting 6–10 μ sections; it has even been possible to obtain serial sections at 5 μ, and in some instances as thin as 3 μ.

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RECENT ADVANCES IN MICROTECHNIC. I. METHODS OF STUDYING THE DEVELOPMENT OF THE MALE GAMETOPHYTE IN ANGIOSPERMS

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ABSTRACT.—**Among methods used for a study of nuclear details in the development of pollen grains, the following were found to be very satisfactory: (1) warming the entire grains in aceto-carmine and then clearing with chloral hydrate; (2) making smear preparations stained with crystal-violet-iodine or iron alum hematoxylin. For paraffin sections, a counterstain with dilute alcoholic erythrosin is often very useful after the usual iron hematoxylin technic.**

A method of making cultures of pollen tubes on slides coated with thin films of sugar agar is described in detail. The tubes can be fixed by immersing the slide in formol-acetic-alcohol and then stained by any desired schedule. Iron alum hematoxylin was found to be the most satisfactory, but the Feulgen reaction is very valuable in such cases where the nuclei are obscured by the density of the pollen tube cytoplasm. Living pollen tubes can be kept under observation by dissolving a small quantity of neutral red or other vital stain in the sugar agar before it is spread on the slide.

For studying stages in fertilization or gametogenesis, styles should be fixed and sectioned only after a preliminary study with iodine-chloral-hydrate or safranin-anilin-blue or aceto-carmine. Once the extent to which pollen tubes grow in a given time in the stylar tissues has been determined, it is possible to fix material with some knowledge of what it is going to show.

Some other methods, that have not been tried by the authors but appear to be valuable, are also briefly described.

Even a cursory glance at the literature on the embryology of the Angiosperms shows that there are many more works on the ovule and embryo sac than on the male gametophyte. Indeed, statements on the latter are often limited merely to a count of the number of nuclei within the pollen grain.

Recent advances in microtechnic have made it possible to follow the whole history of its development, from the time of the first division of the microspore up to the formation of the male gametes,

[1]The senior author expresses his deep sense of gratefulness to Prof. G. Tischler for his kindness and cordiality during the whole period of his stay in Kiel.

with greater precision and ease. It is the object of this paper to summarize this literature here and to describe the more valuable of these methods in sufficient detail to encourage others to make use of them.

Pollen Grain

The customary technic of making paraffin sections of the anthers still holds its place, but it is often possible to make observations of value by simpler means. In a recent paper Wunderlich (1936) writes that she was able to see the generative *cell* in *Haworthia*, *Tulipa*, *Ornithogalum* and *Fritillaria* by just mounting the grains in water without adding any stain whatever. To these we can add, from our own experience, *Hippeastrum hybridum* and *Agapanthus umbellatus*. It is worth mentioning that Meyen (1839), who was the first to see the generative cell, found it in the same genus (*Fritillaria*) and under the same conditions as Wunderlich did nearly one hundred years later.

It must be admitted that such a condition is only rarely met with, but there is another ready method near at hand. All that is to be done is to mount a few pollen grains, fresh or preserved, in a drop of aceto-carmine (made according to Belling's formula), put on a cover glass and warm gently over a flame, adding more aceto-carmine from the sides if necessary (see Heitz and Resende, 1936). Unless the exine is too thick, it is possible to see the generative or sperm nuclei and sometimes even the plasm around them quite clearly. It is often useful to add a few drops of a 10% solution of chloral hydrate (Satina and Blakeslee, 1935), since this clears the cytoplasm from inclusions which otherwise obscure the nuclei or chromosomes. Experience has shown that in some cases when the nuclei or spindle are not readily visible just after warming in aceto-carmine, they can be seen a few days later in the same preparation. If it is desired to save the preparations for some time longer, they can be sealed with a mixture of gum mastic and paraffin.

The following method, used by Warmke and Johansen (1935) to study the first mitosis in the microspores of *Trillium*, may be tried in those cases where the exine is too thick and does not permit an observation of the contents of the grain[2]:

1. Kill in 1 part glacial acetic acid and 3 parts alcohol.
2. Transfer to 1 part 95% alcohol and 1 part conc. HCl for 5–10 minutes. The HCl causes the exine to split and fall away like the shell of a nut leaving the naked protoplast adhering to the slide.

[2]Our own trials, made with pollen grains of some Dicotyledons were entirely unsuccessful. It seems likely that this method will work best with such plants in which the pollen grains have a single prominent furrow.

3. Transfer to a drop of Carnoy's fluid to harden the material.
4. Stain in aceto-carmine in the usual way.

In many cases, where the microspores are surrounded by a viscous fluid in the anther (viz. some Commelinaceae) it is possible to make smear preparations to study the first mitosis in the pollen grain. The usual procedure described by Taylor (1924) answers the purpose admirably, but it is often better to stain in Newton's gentian violet (see Sax, 1931), since this gives greater transparency to the cytoplasm and makes the chromosomes more conspicuous.

It is always useful to check the results by making paraffin sections also, but instead of cutting a large number of anthers at random, it is possible to save time by fixing only material of the required age after a preliminary examination by one of the methods described above. Thus, if it is desired to prepare sections showing the first division of the microspore in *Tradescantia*, we should examine anthers from several flower buds in aceto-carmine. When an anther from a bud does show the required stage, the remaining anthers should be fixed immediately.

Good fixation and a critical stain with iron hematoxylin will reveal the presence of generative and sperm cells in many plants where only nuclei have been reported in the past. A counterstain with erythrosin sometimes makes the effect more sharp. Cooper (1935) who studied the development of the male cells in *Portulaca oleracea* recommends Ehrlich's hematoxylin because of its transparency. In those cases, where the nuclei become obscured by the presence of large amounts of food substances in the pollen grains, the Feulgen reaction often yields fine results since only the nuclei are stained by this method.

Pollen Tubes in Culture

Making cultures of pollen tubes is usually quite easy. Some pollen grains germinate in distilled water without the addition of any other substances (as in grasses). It is also possible to induce germination of pollen grains *in situ* (i. e., in the anther loculus itself) by corking up the flowers in a tube containing some water at the bottom.

Schleiden (1842–43) was the first to try sugar solutions of different strengths for the germination of pollen grains. Kny (1881) was impressed by the similarity of growing pollen tubes and fungus hyphae and began to add gelatine. Trials by other workers demonstrated the superiority of agar-agar[3] and Jost (1907) made his cultures on

[3]It is only in rare cases that agar inhibits the growth of pollen tubes. Thus, Medwedewa (1935) reports that in *Apocynum venetum* the growth of the tubes stopped entirely on the addition of agar to the sugar solution.

slides coated with a thin film of sugar agar. Herrig (1919) germinated pollen grains of *Butomus* and *Echeveria* on agar but he later (1922) cut it up into blocks which were sectioned after embedding in paraffin. This seems to be quite unnecessary since the contents of the pollen tube can be seen quite clearly thru the wall and with sections there is the further disadvantage of having only fragments of the tubes in view and not their entire length.

In recent years Cheesman (1927) records having obtained a good growth of pollen tubes of *Theobroma cacao* on a thin film of sugar agar spread on the slides. He fixed them in Bouin's fluid and used iron hematoxylin for staining.

Trankowsky (1931) perfected the method and tried it on several plants with great success. Since then Wulff (1933, 1934a, 1934c, 1935), Poddubnaja-Arnoldi (1933, 1934, 1936), Safijowska (1935), Fuchs (1936), and others have made considerable use of it. The following outline will, it is hoped, give sufficient information to enable any one to obtain good preparations of pollen tubes showing the male cells and vegetative nucleus, (using, for example, *Impatiens Holstii* or some other species of this genus as a trial object):

1. Culture solution: For every 100 cc. of distilled water, weigh out 1 g. of agar-agar and 2.5, 5, 7.5, and 10 g. of sugar. Plug the tubes with cotton wool and leave in an autoclave or on a water bath till the agar dissolves. These concentrations will suffice for most objects, but sometimes 15–50 g. of sugar may be necessary.

2. Clean slides first with conc. HNO_3 or a mixture of potassium dichromate and sulfuric acid, then rinse in water and rectified spirit and finally wipe them dry with a clean cloth, free from lint. It is necessary that the slides should be absolutely clean because the agar will not spread on dirty slides, and even if it is made to do so it may come off afterwards during the process of fixing and staining.

3. If the culture solution has set, warm it again on a water bath till it becomes perfectly fluid. Flood the right half of the slide with this and then shake off all the surplus sugar agar, so as to leave only a very thin homogeneous film on the slide. If the film is too thick, it will tend to come off at a later stage or it may itself take so much of the stain that the mount will be rendered useless. This film can be spread on half a dozen slides which are then laid flat on a white paper. (A solution containing 1 g. of agar and 2.5 g. of sugar is quite satisfactory for *Impatiens Holstii*.)

4. Let the agar cool for a minute or two and then sprinkle the pollen by means of a sable brush. The flowers must, of course, be kept ready beforehand, each with some half-opened anthers. Immature pollen grains may not germinate. The usual precaution of sterilizing the brush before each attempt with a different plant must not be forgotten.

5. Now keep the slides in a moist chamber maintained at a temperature of 15–22° C. and watch from time to time to see if germination has taken place. In *Impatiens Holstii* 30 or 40 minutes are enough for the division of the generative cell in the pollen tube and it is easy to get many mitotic figures on a single slide. In detailed studies of the division of the generative cell material must be fixed from time to time so as to get all stages.

The aceto-carmine method is particularly useful at this stage and enables a rapid glance of the nuclei in the tube. Just add a few drops of the solution on the slide and put on the cover slip. Warm on a heated brass plate (the time can be determined only by experience) and examine under the microscope. If the nuclei are not clearly seen, add more aceto-carmine from the sides and heat again. Permanent mounts can be made by following the procedure recommended by McClintock (1929) for smears of pollen mother-cells. Steere's method (1931) is not practicable in this case since inverting the slides in hot aceto-carmine loosens the agar film from it. The disadvantage in the aceto-carmine method is that often the vegetative nucleus does not stain at all and the generative plasm around the generative or sperm nuclei is also invisible.

6. For the finest preparations, it is best to fix the cultures and then stain in iron haematoxylin. The slides may simply be immersed in a Coplin jar containing formalin-acetic-alcohol (formalin, 5 cc.; glacial acetic acid, 5 cc.; 50% alcohol, 90 cc.) or Pfeiffer's fluid (equal parts of formalin, methyl alcohol and crude acetic acid). Nawaschin's fluid or chrom-acetic acid may also be used, especially if gentian violet is to be used for staining. The only disadvantage is that the pollen tubes do not hold so well on the slides after fixation in aqueous fluids and sometimes they burst during the process of fixation. The latter difficulty can be overcome (as suggested by Trankowsky) by first fixing for 2–3 minutes in a solution containing the same percentage of sugar that was used in the culture. After this preliminary killing, the slides can be transferred to another jar containing the fixing fluid made up according to the original formula. The time of fixation should be about three hours.

7. Wash the fixative with water or alcohol as the case may be. Only half a dozen changes will be enough. Let the slides remain in the jar and allow a gentle stream of water to flow in from one side. Careless handling at this stage may cause the agar film to get lose from the slides.

8. For staining follow the usual procedure with iron alum hematoxylin. About 10 minutes of mordanting and 10 minutes of immersion in hematoxylin will be sufficient. Differentiate in a saturated aqueous solution of picric acid or 2% iron-alum. Use distilled water for washing and pass thru 30, 50, 70 and 90% alcohols to absolute alcohol. Before passing into pure xylol it is advisable to use one grade of a mixture of absolute alcohol and xylol. Mount in thin Canada balsam. The generative nucleus will be well stained and its cytoplasm will be distinguishable from the cytoplasm of the tube.

Newton's gentian-violet-iodine method will also give good results if the tubes are broad and the nuclei large, but it is necessary that fixation be carried out in a fluid containing chromic acid. The disadvantage of this method is that the cytoplasm around the generative nucleus usually remains indistinguishable from the general cytoplasm of the tube.

The Feulgen reaction (Wulff, 1933) can be made use of in special cases to ascertain whether the vegetative nucleus, which is often difficult to see even after staining with hematoxylin, is still present or has actually degenerated (or fragmented). Many of the pollen tubes get washed away during the period of hydrolysis in warm HCl, but when sufficient care is exercised some can always be retained on the slide and give valuable results for comparison.

Vital staining. This is of some importance since it enables observations on living pollen grains and pollen tubes. Wóycicki (1926a, 1926b) was the first to show that in *Haemanthus Katharinae* the plasm of the generative cell contains a vacuome which can be seen after staining with neutral red. Since then it has been seen in several other Monocotyledons (Wulff 1933) and in such cases this method can be used not only to demonstrate the presence of the generative plasm but also in following the passage of the generative or sperm cells, which stain red when pollen tubes are grown on a sugar agar substrate containing a small quantity of neutral red in solution. Similar results have been reported with chrysoidine (Wulff, 1934b) but we do not yet have enough observations of this kind to say in how many cases it will actually succeed. A brief mention may also be made of the work of Breslavetz who used "Janusschwarz" to stain pollen tubes of *Cylamen* and found the sperm nuclei to be surrounded by a lighter zone while the mitochondria, which were seen only in the vegetative plasm, took a black stain. Finally there are some exceptionally favorable plants like *Agapanthus umbellatus* and *Hippeastrum hybridum* where the generative cell can be seen in the pollen tube without any staining whatever.

Pollen Tubes in Style

While pollen tubes can be grown quickly in the way described above and all stages in the division of the generative cell (unless it has already taken place in the pollen grain) obtained by fixing them at regular intervals, this method can not entirely take the place of observations made on tubes growing in the style. As shown by one of us (Wulff, 1935) the mechanism of division of the generative cell

may show important differences under the two conditions and a comparison is always useful.

In this case also, a great deal of time and labor can be saved by making some preliminary observations before fixing the material for embedding it in paraffin. In the case of slender styles, it is possible to determine the presence of pollen tubes merely by staining the entire styles in iodine and clearing with chloral hydrate. Those parts of the tubes which contain starch stain black, while the starch-free portions stain dark yellow. If permanent preparations are desired, the entire styles can be fixed in formalin-acetic-alcohol or Carnoy's fluid and stained in eosin after which they can be dehydrated, cleared and mounted in balsam. A combination of safranin and anilin blue is also suitable since this stains the callose of the wall of the tube and makes it conspicuous. Better still is to stain in aceto-carmine, dehydrate with alcohol, clear with clove oil, then rinse with xylol and finally mount in balsam. Poddubnaja-Arnoldi, Steschina and Sosnovetz (1935), who tried this method in their work on *Scorzonera tausaghyz*, report that the pollen tubes alone took the stain while the other tissues of the style remained practically colorless, so that it was possible to trace their course very easily and in some cases even the sperm nuclei were visible.

In the case of thicker styles, free-hand sections can be made and stained by one of the methods given above. Nebel (1931) recommends crushing the styles between two slides and then staining with 5 mg. of lacmoid and 5 mg. of martius yellow dissolved in 10–15 cc. of water. Anderson (1936) smeared the styles on a slide at various intervals after pollination and then stained with crystal violet. Pollen tubes could thus be traced right down to the micropyle, after which the ovaries were fixed at 2 minute intervals until fertilization had occurred.

Another method (Schoch-Bodmer, 1932), which can be tried for long styles, is to pollinate the stigma, cut it off with a small part of the style attached to it and keep the piece in a moist chamber. The pollen tubes will emerge out of the cut end after some time. The conditions requisite for success are: a clean cut surface, without a blocking of the intercellular spaces and a proper degree of moisture and temperature. This gives an approximate idea of the rate of growth of the pollen tubes and by measuring the total length of the style in open flowers it is possible to make a rough prediction about the approximate time that will be taken by the tubes to reach the ovary. It is of interest to add that Buchholz, Doak and Blakeslee (1932) made some careful experiments and found that it is possible to shorten

the length of the style by removing a piece from the middle and joining the two cut ends. Even when parts of two different styles were joined and properly held in place, many pollen tubes could pass thru the wounded area.[4]

The presence of the pollen tubes once determined, it is possible to fix material with some knowledge of what it is going to show. Thus, if the division of the generative cell is to be studied, it is best to fix styles at different intervals soon after the pollen tubes have begun to germinate (a preliminary study of cultures stained with aceto-carmine may often indicate the time of the division of the generative cell rather precisely). If the object is to study stages in fertilization, fixation should begin at very close intervals after the pollen tubes have reached the base of the style.

Small and thin styles offer no special difficulty in fixation and can be dropped whole into the killing fluid, but it is best to cut up or split the larger ones and use an exhaust pump to facilitate infiltration. Nawaschin (1910), in his excellent work on *Lilium*, writes that he injected the killing fluid into the styles.

For observation of cytological details there is nothing better than the customary iron hematoxylin technic, but a counterstain with dilute alcoholic fast green increases the visibility of the pollen tubes. If they are relatively broad and the material has been fixed in one of the chromic acid fluids,[5] Newton's gentian violet and iodine will also give good results. Sieben (1913) gives 2 mixtures of the Pianeze stain, and in his work on *Asclepias cornuti*, Finn (1925) recommends this as the best. Without giving further instances it may be stated that some plants react favorably to one method and some to another and the investigator has to find out for himself which is best for his particular needs—first, for locating the pollen tubes and thus determining the approximate time for the fixation of the styles and then for a critical study of the contents.

Finally, a brief mention may be made of cases when it has been found possible to dissect out the pollen tubes from the style and then

[4]Jost (1907) had used an essentially similar method to bring about the opposite effect of lengthening the style. In *Hippeastrum* the style was cut off at the base a day after pollination and then placed in a moist chamber with its lower end in close contact with the stigma of another flower. A week later the tubes had travelled into the second style. The same was accomplished in *Lilium martagon* by bringing the cut surface of the first style in contact with the upper end of the cut surface of a second style. In this case the second stigma was removed since it caused difficulties in the passage of the tubes.

[5]Artschwager and Starett (1934) report that in *Beta vulgaris* the pollen tubes could be located with difficulty in material fixed with Carnoy's fluid but stood out distinctly when Nawaschin's was used.

stain them—an operation whose success depends to a large extent on the structure of the style. Sawyer (1917) found that in *Iris versicolor* each of the 3 branches of the style is traversed by a longitudinal groove covered by 2 overlapping outgrowths, thus forming an inclosed canal with the pollen tubes inside it. By removing the covers of the groove, she could expose the canal containing the tubes and remove them with needles. In some cases it is possible to split the style longitudinally by beginning at the stigma and carrying the cut downward with a pair of fine needles. The pollen tubes can then be exposed by spreading the cut surfaces apart and laying on a clean slide. Further details will be found in the papers by Chandler (1931) and Buchholz (1931).

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NOTES ON TECHNIC

A Cellophane Embedding Box.—A new paraffin embedding technic which may be of interest to others is being successfully used in this laboratory. It involves the use of embedding boxes made of No. 450 M. T. cellophane instead of paper boxes. They are made by cutting and folding the material just as paper boxes are made. This new method has these advantages:

1. The fixed material is dehydrated, cleared, and paraffin is added, without leaving the cellophane box.
2. The transparent box allows the smallest material to be observed easily under the dissecting microscope.
3. The cellophane separates from the paraffin cleanly.

Pipets with annealed tips are recommended for handling small material when using this technic.—F. F. Ferguson, University of Virginia, University, Va.

A Technic for Staining Cells With Sudan III in a Water Phase.—Since Sudan III is not soluble in water its use is attended with some difficulty. It is generally employed in a solution of alcohol, or alcohol and glycerin. There is an objection to these solvents, especially when one wishes to study both the lipidic features and the hydration properties of the intravacuolar spheres. A solvent is needed which will carry Sudan III in an aqueous phase into the cell, without the previous use of killing agents.

Spherical inclusions of lipoidic material in the cell vacuole are among the most important indices of impaired physiological activities of the plant cell. We have shown that the hypoplastic cells of orange leaves affected with mottle-leaf commonly contain these highly refringent spheres of phytosterol material, mainly in the epidermal or adjoining palisade layers.[1] Similar bodies are to be found in cells of various plants in the families of Solanaceae and Compositae when affected with the "spotted wilt". These spheres stain readily with fat soluble dyes, specifically with Sudan III; or they may be stained gray with osmic acid in low concentration, or blue with nascent indophenol blue, formed as a suspension in a mixture of alkaline solutions of paradiphenylamine hydrochloride and thymol.

We have found it possible to study the physical properties of these spherical inclusions as a lipid phase in contact with the aqueous phase of the vacuolar sap by the method hereinafter described.

[1]Reed, H. S., and Dufrenoy, Jean. 1935. Modification in cell structure accompanying mottle leaf of the orange. Amer. J. Bot., 22, 311–28.

Methylal was used to dissolve Sudan III, and to carry the Sudan III in the form of a suspension into water.

A strong solution of Sudan III was first prepared in 5 cc. of methylal. This was poured into a small vial and 10 to 20 cc. of water added. The water first mixes with the methylal, but in a few minutes the liquids separate, leaving the lower, light orange phase containing water + methylal + Sudan III and an upper phase containing methylal + Sudan III + water.

When free-hand sections of tissue whose cells contain intravacuolar spheres (for instance, leaves of *Callistephus sinensis* affected by the virus of "spotted wilt") were immersed in the liquid, they either sunk to the bottom of the vial, or floated at the interface. In either case they were in contact with a water phase from which they absorbed some Sudan III. As they absorbed dye from the water phase, dye entered from the upper concentrated solution.

Sections were transferred to a slide at the end of 30 minutes and studied under the microscope. The intravacuolar spheres of lipoid material were stained bright orange.

In the course of several hours the methylal may become hydrated to an extent that Sudan III no longer stays in solution, but it remains in solution long enough for the technic above described.—J. DUFRENOY, Station de Pathologie Végètale, Pont-de-la-Maye, Bordeaux, France and H. S. REED, University of California, Berkeley, California.

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi — *Abstract Editor*

BOOK REVIEWS

GAGE, SIMON HENRY. **The Microscope.** 16th ed. 6 x 9½ in., 617 pp. Cloth. 311 illustrations. Bibliography. Index. Comstock Publishing Co., Ithaca, N. Y. 1936. $4.00.

The sixteenth edition of this well-known book is published 4 years after the preceding. Altho the price has not been changed, the text material of this edition has been increased by 25 pages; also the general appearance of paper, print, and binding has been improved. The major new addition is a chapter on micro-incineration. It includes the technic of reducing sections to ashes in such a way as to permit identification of the various inorganic materials in position; also it offers suggestions as to the best optical equipment suitable for such a type of work. A section discusses fully the coating of mirrors by the aluminum vapor method and their superiority over quartz prism reflectors for ultraviolet microscopy. The bibliography has also been brought up to date.—*J. A. de Tomasi.*

MICROSCOPE AND OTHER APPARATUS

FOOTER, A. W. **Simplified preparation of microscope cross hairs.** *Science*, **84**, 490. 1936.

For some time the author has been using spider silk for cross hairs on microscope oculars; but because of the difficulties in obtaining and using this material, he now proposes an improved procedure. The proposed method relies upon the use of a commercial waterproof adhesive (not specified, but assumed to be pyroxylin). A small globule of this cement is taken on the end of a probe and touched to the surface of the diaphragm of the ocular, after removal of the upper lens. Pulling the probe away some 9–12 in. leaves an extremely fine and elastic thread attached to one side of the diaphragm. The opposite side of the diaphragm is then moistened with the same adhesive and the thread from the first drop of the substance is caught a little short of the length necessary to cross to the other side; it is then stretched out to the requisite extent and secured on the opposite side of the diaphragm by means of the second drop of adhesive.—*H. J. Conn.*

KNISELY, M. H. **A method of illuminating living structures for microscopic study.** *Anat. Rec.*, **64**, 499–524. 1936.

This paper describes a quartz rod method of illumination for studying living organs, with especial reference to circulatory changes including capillary blood flow. Fused quartz rods, if meticulously clean and free from wrinkles and bubbles, conduct the light waves without great loss even around curves to a bent up delivery tip placed beneath the organ to be studied. The fact that quartz is a poor conductor of heat helps in preventing too great conduction to or from the tissues studied. The light source is generally a tungsten filament projection bulb, air cooled and used without condenser lens. At times a condenser made in the form of a glass funnel, externally silvered, was used and the receiving end of the quartz rod placed adjacent to the narrow end of the funnel. The angle at which the light rays enter the quartz rod should be greater than the critical angle of fused quartz so that internal reflection will keep the rays within the rod and

send them toward the delivery tip. The quartz rod is made in two sections so that filters may be inserted between the source and delivery ends. Rack and pinion is used for movements of the delivery tip, and 2 adjustable clamps for the ends nearer the light source. Adequate drawings show the arrangement of the parts. The animals studied are mounted on a 3-legged table free from the microscope. This can be moved without disturbing the delivery tip of the quartz rod. The end of the rod should not touch the tissue. The tip is further cooled by a stream of Ringer's solution against its side. The control of vibration in the apparatus is described. There follows a discussion of the efficacy of the method to demonstrate normal processes arrived at by testing animals under varying conditions of anaesthesia and illumination. Intense light, if properly cooled, did not seem to alter capillary circulation. A summary of the advantages and limitations of the method is given.—*S. I. Kornhauser.*

SCOTT, G. H. and WILLIAMS, P. S. **A simplified cryostat for the dehydration of frozen tissues.** *Anat. Rec.*, **66**, 475–81. 1936.

This apparatus is constructed entirely of metal. The tissue chamber is immersed in a 3-gallon pyrex vacuum flask containing solid CO_2 and butyl alcohol, which produces a temperature of —78° C. From the upper part of the metal casing containing the tissue chamber is an outlet to a Cenco Hyvac pump. A mercury regulator is connected with an alcohol chamber placed directly above the tissue chamber. The alcohol chamber is wound with an electrical heating element by which the chamber may be warmed during the process of dehydration. By means of the regulator, the temperature of the tissue chamber may be varied between —70° C. and 0° C., the higher temperatures facilitating the removal of the water vapor from the frozen tissues. The cost of operating the apparatus in cool weather is less than 20 cents for 24 hr. A sectional mechanical drawing of the apparatus is given.—*S. I. Kornhauser.*

SHINN, L. E. **Protection of eyepieces.** *Science*, **84**, 400. 1936.

To prevent upper lenses of eyepieces from getting soiled or dusty, a circular cover slip of 20–25 mm. diameter is a convenient cover, when casing rim allows it. It is easily removed and does not interfere with vision.—*J. A. de Tomasi.*

PHOTOMICROGRAPHY

KOCH, W. **Eine einfache Vorrichtung zur Mikrophotographie und zur Photographie kleiner lebender Objekte.** *Zts. wiss. Mikr.*, **53**, 37–42. 1936.

This is substantially a report on the adaptation of a well-known miniature camera to photomicrography. The camera is the "Exacta" made by the Ihagee-Kamerawerke of Dresden, Germany. Its outstanding features are a reflecting mirror for focusing and a focal shutter. Additional equipment suggested consists of a tube extention, a hinge piece connecting the camera to the microscope, and a ring diaphragm.—*J. A. de Tomasi.*

MICROTECHNIC IN GENERAL

EDMONDSON, W. T. **Fixation of sessile Rotatoria.** *Science*, **84**, 444. 1936.

Fixation in a life-like manner of sessile Rotatoria is extremely difficult. The following procedure is suggested: By means of a pipet take the rotifer attached to a small part of its host plant and transfer it to a watch glass. When animal is extended, pour onto it 1–2 cc. of boiling Zenker's solution. Rinse immediately in water. The technic proved to be satisfactory with several genera except *Floscularia* and *Limnias*.—*J. A. de Tomasi.*

HOERR, N. L. **Cytological studies by the Altmann-Gersh freezing method. I. Recent advances in the technique.** *Anat. Rec.*, **65**, 293–313. 1936.

A history of the freezing drying method is first sketched. The author's present methods are then given and finally a statement of the advantages and disadvantages of the method together with cytological illustrations.

Tissues are immersed in iso-pentane chilled to —195° C. by liquid nitrogen. In 15 sec. a piece 5 mm. thick is frozen without producing visible gas bubbles.

For large pieces of tissue 200 cc. of iso-pentane should be used. Liquid nitrogen is the safest refrigerant. Animals dehydrated by starvation for several days show fewer ice crystal artefacts. In the dehydration of frozen tissues a temperature of —20° C. did not prevent formation of ice crystals which resulted from the concentration of salts of tissues during dehydration. In general, —33° C. gave good cytological pictures. For some tissues —60° C. was used. The temperature must be reduced slowly to the dehydrating temperature. In general, rapid dehydration is best and a triple stage mercury diffusion pump, backed up by a Hyvac oil pump, was used. A McLeod gauge and a discharge tube indicated when dehydration was complete. Phosphorus pentoxide aided in obtaining high vacuum. From 0.5–1% of water remains in the tissue and can be removed by desiccation in $CaCl_2$ at 37° C. Paraffin embedding of dehydrated tissues is held injurious as paraffin and petroleum ether dissolve the highly dispersed lipoids in the protoplasm. Free-hand sections 15–20 μ can be cut of most tissues. These may be cleared in 1 min., or less in vacuo in a drop of paraffin oil or anhydrous glycerin.

Enzymes are not destroyed by freezing and drying. The main advantage is instantaneous fixation or cessation of chemical activity. Thus the spleen will show structures only seen in life and not seen after ordinary fixation. Vacuoles of various sizes and form are always formed but rapid freezing reduces these to a minimum. The smallest vacuoles are spherical and are believed to be caused by gases making their exit from the tissues.—*S. I. Kornhauser.*

IWANOFF, X. **Über das Aufkleben von Gefrierschnitten.** *Zts. wiss. Mikr.*, **53**, 48–9. 1936.

Frozen sections can be made to adhere to glass by the following procedure: Smear the cover slip with a 2:1 albumen-glycerin mixture and dry for 24 hr. in the upper shelf of the paraffin oven; if desired to avoid this delay, a 1:1 mixture of albumen (or blood serum) and 5% formalin can be used immediately. Transfer section to the slip. Support a few cover slips, sections right side up, with a glass slide. One ordinary slide will hold 4 average size cover slips. Cover with a strip of filter paper the size of the slide, put on another slide, press down, remove upper slide, fix the section by wetting with 1–2 drops of abs. alcohol or 40% formalin, replace second slide and press down gently for 30 sec.—*J. A. de Tomasi.*

WERCH, S. C. **The use of liquid air in cooling knives and of gelatin for mounting in frozen section technic.** *J. Lab. & Clin. Med.*, **21**, 1309. 1936.

In connection with the frozen section technic two practical hints are suggested. The microtome blade can be immersed without damage in liquid air in a thermos bottle just before using. The effect of this treatment lasts about 5 min. The use of 10% gelatin instead of glycerin eliminates most of the trouble due to bubbles.—*J. A. de Tomasi.*

DYES AND THEIR BIOLOGICAL USES

HOLLBORN, K. **Hämatoxylin (Vereinfachte und verbesserte Hämatoxylin-Simultanfärbungen.).** *Zts. wiss. Mikr.*, **52**, 184–9. 1935.

Hematoxylin is a crystallin product obtained by a water-ether extraction of the hardwood of *Hämatoxylon campechianum*. The addition of Fe, Cr, and Al ions changes it to lakes which behave like basic compounds, displaying a specific affinity for the nucleic acid of the nucleus. The following directions are given for the preparation of a number of hematoxylins and hematoxylin compounded stains, all made from Bayer's standardized hematoxylin.

Hämatein und Hämatein-Ammon: Use this product by dissolving 1 g. in 1000 cc. 5% alum by heating. Cool and filter. Preserve by adding 10% hartosol, methyl or ethyl alcohol.

Hämalaun: Dissolve 5 g. in 100 cc. hot dist. water. Cool, filter and preserve as above.

Eisenhämatoxylin "H": Dissolve 2 g. in hot dist. water. Cool and filter. Stain sections 20–30 min. Rinse in water and alcohol. Clear and mount. This can be followed by *Van Gieson-Elastin-Farbstoff "H"* for a multicolor stain.

Nucplascoll: Dissolve 2 g. in 100 cc. hot dist. water. Cool, filter and preserve as above. Treat sections as with Fe hematoxylin. Nuclei stain black; collagenous fibers, green; muscles, red; blood and hairs, yellowish.

Hämatoxylin-Eosin "H": Preparation and staining as with nucplascoll. Nuclei appear black; the eosin gives a multicolor stain.

Hämatoxylin-Sudan "H" Lösung: Supplied only in solution. A simple application will stain nuclei black and the fat red. Blood films or frozen sections can be stained 1 hr. in closed jar. Rinse in 70% alcohol, water and mount in glycerin, glycerin-gelatin, glycerin-rubber, or gelatin-balsam.

All these stains, for which simpler manipulation and saving of time is claimed, are products of the firm Dr. K. Hollborn & Söhne, Leipzig.—*J. A. de Tomasi.*

HOLLBORN, K. **Karmin und Cochenille. (Vereinfachte und verbesserte Karmin- und Kernechtrot-Simultanfärbungen.)** *Zts. wiss. Mikr.*, **52**, 209–13. 1935.

Among many dyestuffs used in staining histological material only one substance is derived from an animal source. It is cochineal or powdered wingless females of the nopal insect (*Coccus cacti*), grown on specially established plantations in Mexico, Brazil, Peru and Java. This powder has a content of about 10% carminic acid. Neither cochineal nor carminic acid have found as wide an application as carmine, a mixture of carminic acid, clay, calcium, and albuminoids. The author lists a number of carmine combinations he worked out in the last decade.

(1) *Pikrokarmin neu I "H":* Stains nuclei red, remaining tissue yellow. Does not require differentiation. Preparation: dissolve 4 g. of dyestuff in 100 cc. hot dist. water. Cool and filter. Stain 30 min.

(2) *Pikrokarmin-Anilinblau "H":* The addition of anilin blue adds blue-stained connective tissue to the picture given by No. 1. Preparation: dissolve 2 g. of dyestuff in 100 cc. hot dist. water. Cool and filter. Stain 30 min., wash with dist. water, pass thru alcohol and xylene into Canada balsam, free from essential oil.

(3) *Multikolor Solution:* Stains nuclei red; elastin brown; erythrocytes and keratin yellow; fat green.

(4) *Nucplastin:* A combination of carmine, acid green, and "elastin stain". Preparation: dissolve 4 g. of dyestuff in 40 cc. hot dist. water on a water bath, 10 cc. glycerin, 50 cc. hartosol or alcohol. Cool and filter. Stain 30 min. in covered jar and refilter before each application. Rinse with hartosol or alcohol; xylene, Canada balsam "H", or "Caedax".

"*Kernechtrot standardisiert, Bayer*" is a synthetic product intended as a substitute for, and claimed to be superior to carmine as a red nuclear stain. It is an anthraquinone dye to be used in 0.1% concentration in 5% $Al_2(SO_4)_3$ solution dissolved by boiling. "*Kernechtrot-Kombination H*" is a compounded product derived from it and gives simultaneous staining of nuclear and connective tissue. Prepare by dissolving 2 g. in 100 cc. hot dist. water, cool and filter. Stain 5–10 min.

"*Kernschwarz H*" is a black water soluble dye used as a nuclear stain for plant material. Make a 4% solution in dist. water and heat it in a boiling water bath for 10 min., cool and filter. Stain 20–30 min. "*Kernschwarz-Kombination H*" is a combination of this black dye with acid fuchsin and picric acid. When prepared like No. 2 above it gives, with animal material, a Van Gieson stain and black nuclei.—*J. A. de Tomasi.*

HURD, C. D. and SCHMERLING, L. **Alkenyl derivatives of fluorescein.** *J. Chem. Soc.*, **59**, No. 1, 1937.

Allyl, pentenyl, and hexenyl derivatives of fluorescein were synthesized. These involved the "ether ester," "diether," "monoether" and "monoester" types. They have very low phenol coefficients.—*Roy L. Mobley.*

PREISLER, P. W. and HEMPELMANN, L. H. **Oxidation-reduction potentials of derivatives of thioindigo. I. Thioindigo tetrasulfonate.** *J. Chem. Soc.*, **58**, No. 11, 1936.

The preparation and tests of thioindigo tetrasulfonate are given. The constant $E_o = 0.409$ was found for the system and the first pK of the reductant was 4.5.

The substance is suitable for use only where acidity is greater than pH 2.5.—*Roy L. Mobley.*

ROMEIS, B. **Neue Untersuchungen zur Fettfärbung mit Sudan.** *Centbl. allg. Path.*, **66**, 97–104. 1936.

Sudan III (Hollborn) of recent manufacture contains chiefly Sudan red, whereas older Sudan III contained considerable Sudan orange and yellow. The new dye is used as follows. Dissolve 1 g. Sudan III in 100 cc. 80% alc. by bringing to boiling in a water bath. Provide the flask with rubber stopper and reflux tube 1 m. long by 6–8 mm. diameter. Let cool to 20° C. then cool further in running water for 30–60 min. Filter next day and store in a tightly stoppered Jena glass flask. This is the stable stock solution. To make the staining solution, filter 20 cc. of stock solution and dilute with 20 cc. dist. water. Put into centrifuge tubes and centrifuge 30 min. Pour off the supernatant fluid, keep well stoppered and filter before use. Stains in 4–5 hr. at 28° or 12–16 hr. at 19–20° C.—*H. A. Davenport.*

T'UNG, T. **Photodynamic action of methylene blue on pneumococcus.** *Proc. Soc. Exp. Biol. and Med.*, **35**, 399–400. 1936.

A virulent culture of pneumococcus Type III was grown in Avery's medium for 18 hr. and mixed in a Petri dish with saline saturated with methylene blue in a ratio 9:1. Half was exposed, over a cooling unit, for 45 min. to an electric light of 100 candle power at a distance of 10 cm. The organisms were washed and incubated at 37° C. together with a control suspension preserved with 0.4% formalin. Organisms treated with dye and with light, even after 6 months, were typical in morphology and in reaction to Gram's stain. Smears were treated with alcohol for 2 hr. before staining. Using pneumococcus Type I, dye-treated organisms resulted in better agglutinin titers, and gave better protection in mice than did control suspensions.—*M. S. Marshall.*

ANIMAL MICROTECHNIC

AMPRINO, R. **Un perfectionnement technique à la méthode d'Achucarro, pour les fibrilles grillagées, avec quelques considérations sur les méthodes de coloration élective du tissu conjonctif.** *Bull. d'Histol. Appl.*, **13**, 223–34. 1936.

This modification of Achucarro's method for reticular tissue, based upon impregnation with Ag, aims to eliminate excessive staining of cells and to sharpen its selectivity for the finer fibrils. The following procedure is recommended: Fix as long as possible in 12% formol or alcohol-formol. Embed in celloidin, cut and affix 6–7 μ sections. Dissolve celloidin and wash in dist. water. Heat slides 30–40 min. in 1–2% tannin in alcohol at 55–58° C. Rinse in dist. water and pass thru 3 jars of Ag-diamine hydroxy-chromate solution, 3 cc.; dist. water, 10 cc. Prepare the Ag solution as follows: To 2 cc. of 20% H_2CrO_4 add 4–5 more drops of 10% KOH than are necessary to obtain a change of color from reddish to lemon yellow; pour 1 cc. of the chromate solution into 3–4 cc. of 10% $AgNO_3$; wash the precipitate thoroly with dist. water and dissolve in NH_4OH (concentration not specified). Sections must come out of the third jar slightly yellow in color. Rinse 30–40 sec. in dist. water without stirring. Reduce in 30% formol. Each fibril stands out quite clearly against a very light background and artefacts from Ag precipitation should not disturb the preparation.—*J. A. de Tomasi.*

BERBLINGER, W. and BURGDORF. **Neue Färbemethode zur Darstellung der Gewebsbestandteile der Hypophyse des Menschen.** *Endokrinologie*, **15**, 381–8. 1935.

The new technic described for the demonstration of the various components of the hypophysis consists of the following steps: Fixation in 4–10% aq. or alc. formalin; hardening and dehydrating in graded alcohols; chloroform; paraffin. Stain sections, after removal of paraffin, 2–24 hr. in an alc. solution of cresofuchsin (0.5–1%); wash briefly in dist. water; then in alum carmine for 3 hr.; rinse in dist. water. Differentiate and stain the acidophil cells for 5 min. in the following solution: 2 g. orange G in 100 ml. of 1% phosphomolybdic acid; rinse in dist. water, place in 5% phosphomolybdic acid for 2 min., dry with blotting

paper, stain for 10–20 min. in: (dist. water, 100 ml.; water soluble anilin blue 0.5 g.: bring to boil, filter cold and dilute filtrate with 2 parts dist. water), rinse in dist. water, differentiate in 75% alcohol until stain no longer washes out. Dehydrate, clear, mount. Chief cells, blue to gray; pregnancy cells, blue with small vivid yellow granules; basophiles with coarse reddish-blue granules; epithelium of the pars intermedia and pars tuberalis, variable; collagen fiber, intensive blue; glia fibers, blue-gray; axons occasionally nearly black. (*Cited from* Ber. wiss. Biol., **35**, 354. 1936. Original not seen; hence the indefinite nature of some of the above dye solutions.)—*J. M. Thuringer.*

BODIAN, D. **A new method for staining nerve fibers and nerve endings in mounted paraffin sections.** *Anat. Rec.*, **65**, 89–97. 1936.

Sharp and selective staining of the nerve elements of the central and the peripheral nervous systems can be accomplished by impregnation of mounted paraffin sections with protargol (silver albumose, Winthrop Chemical Co., New York) and reduction of the silver with hydroquinone. A small amount of metallic Cu must be added to the protargol bath. Mounted sections can be stained in less than 24 hr.

Fixation: With perfusion bottle at height of 6 ft. perfuse rapidly thru the heart with a minimum amount of saline (100 cc. for a cat), followed by 500 cc. of 80% alcohol. Remove central nervous system and place in 95% alcohol for 7–28 days at 37° C. Change alcohol frequently. Perfusion can be started during anaesthesia with chloral hydrate or the barbiturates, or after killing with illuminating gas. If vessels of brain contain much blood, perfusion has not been complete.

Embed in paraffin. Section. Mount. Remove paraffin with xylene and run sections thru abs. alcohol and 95% alcohol to dist. water. Place sections in a solution of 1% protargol containing 4–6 g. metallic Cu per 100 cc. of solution for 12–48 hr. at 37° C. Wash in dist. water. (The protargol solution can be used only once.) Reduce for 10 min. in: hydroquinone, 1 g.; Na_2SO_3, 5 g.; dist. water, 100 cc. Wash thoroly in dist. water. Tone for 5–10 min. in: $AuCl_3$, 1 g.; glacial acetic acid, 3 drops; dist. water, 100 cc. Wash in dist. water. If sections do not have a light purple color, place in 2% oxalic acid until a definite purplish tinge appears (usually 5–10 min.). Wash in dist. water. Place in 5% $Na_2S_2O_3$ for 5–10 min., to remove residual Ag salts. Wash thoroly in dist. water, dehydrate, and mount in balsam. Sections of formalin fixed material should be placed for 24 hr. in a solution of 4% NH_4OH in 95% alcohol before being placed in the protargol solution.—*James B. Rogers.*

COVELL, W. P. **A cytologic study of the effects of drugs on the cochlea.** *Arch. Otolaryng.*, **23**, 633–41. 1936.

This paper deals with the cytological alterations induced in the cochlea by the administration of such toxic agents as quinine and sodium salicylate. In this procedure the step of decalification is eliminated. Two groups of 16 animals each, including rabbits, guinea pigs and white mice, were given daily for 2–8 weeks, subcutaneous injections of 2.5% quinine bisulfate and 2.5% sodium salicylate, respectively, (200 mg. per Kg. of body weight). A third group was kept as controls. Each animal was anesthetized and perfused with warm saline and with 1:10 formol U. S. P. The histological procedure followed was the one described by Guild (Guild, S. R. War deafness and its prevention: Report on the labyrinths of the animals used in testing of preventive measures. J. Lab. & Clin. Med., **4**, 153, 1919). Two series of slides, from each fifth section, of 10 μ are stained with hematoxylin-eosin and, with a modified Van Gieson stain. In the guinea pig it is found that the mitochondrial fixatives, such as Regaud's solution, sufficiently decalcify the thin bone of the cochlea to permit sectioning in paraffin. Proceed as follows: Dissect one cochlea free, fix 3 days in Regaud's, changing daily, transfer to 2.5% $K_2Cr_2O_7$ for 4 days and change daily. Fix the other cochlea 24 hr. in Bensley's A. O. B. liquid. A small opening in the apex of the cochlea and also in the round window permits rapid penetration of fixative. The A. O. B. liquid does not cause distortion or vacuolation in the cells of the stria vascularis, nor of spiral ganglion cells. It is concluded that quinine and salicylate produce drastic alteration and damage to mitochondria and ground substance of cells of those structures.—*J. A. de Tomasi.*

GRIFFITH, J. Q., ROBERTS, E. and JEFFERS, W. A. **A staining technic for blood in spinal fluid.** *J. Lab. & Clin. Med.*, **21**, 1208–10. 1936.

In order to differentiate between a fresh hemorrhage and one more than a day old, the following procedure is suggested:

Dry the spinal fluid smear thoroly for 15–30 min. No fixative is necessary. Immerse 20 min. in 50% alcohol saturated with Sudan III. Rinse 3–4 min. in several changes of dist. water and blot dry. Mount in glycerin. Slides are not permanent as the stain fades in a day or so. Red cells of fresh hemorrhage in cerebrospinal fluid stain uniformly pale yellow; 24-hour old cells show unstained centers and ring-like peripheries.—*J. A. de Tomasi.*

HAUSDORF, G. **Über eine histologische und haematologische Schnellfärbemethode.** *Deut. Aerzte-Zeitung, No.* **382**, **8**, 1–4. (Published by DAZ-Verlag Richard Parske, Berlin SO 16.)

A 2% solution of *Nucplascoll* (Hollborn) stains histological preparations and tissue sections within 1 hour. (The name is merely a trade designation and does not indicate the composition of this compounded stain.—*Editor*) Cut pieces of tissue as thin as possible, fix 15 min. in 10% formol at 40–45° C. Rinse in tap water or saline, cut with the freezing microtome. Pass thru water or saline and transfer to a glass containing the staining solution. Heat to vapors (1–2 min.), pass directly into 80% alcohol and finish as usual. Nuclei appear red blue; collagenous connective tissue, brilliant green; muscles and vessels, yellowish-red.

Nucplascoll as blood stain: Fix by flooding slide for 3–4 min. with pure methyl alcohol. Wash in tap water. Stain 30 min. to 2 hr. in 2% *Nucplascoll*, or by heating to light bubbling on a Bunsen flame. Rinse in water and blot dry. Erythrocytes appear green, nuclei of leucocytes, deep blue.—*J. A. de Tomasi.*

HOLLBORN, K. **Eine neue Universal-Färbemethode.** *Zts. wiss. Mikr.*, **53**, 46–7. 1936.

Blockman modified the Van Gieson stain by substituting triphenylrosanilin-sodium trisulfonate (methyl blue?) for acid fuchsin, thus staining the connective tissue blue. This modification, along with chrom-hematoxylin has been embodied into the new universal *Chrom-Hämatoxylin "H"* and *Blochmann-Elastin-Farbstoffe "H"* stain. Dissolve 2 g. chrom-hematoxylin in 100 cc. tap water for 5–10 min. in a boiling water bath. Cool and filter. Stain sections 15 min. Rinse in water and in hartosol or alcohol. Stain 1 hr. in Blockmann-elastin stain under a cover (0.5 g. in 100 cc. of 70% alcohol or hartosol; filter). Rinse with alcohol, clear and mount. *Kernechtrot* can be substituted for chrom-hematoxylin, as a nuclear stain, for 5 min. The results are similar to the Azan stains, with the added advantage of a sharp dark brown contrast coloring for the elastic fibers.—*J. A. de Tomasi.*

JONES, R. L. **On the preparation of microscopic sections for making fiber counts of nerves containing unmyelinated fibers.** *Anat. Rec.*, **65**, 247–54. 1936.

Myelinated and unmyelinated axones of the vagus nerve can be stained in sections made by a combination of the three following procedures. More distinct staining is obtained than by the usual pyridine silver methods. Fixation and silver impregnation follow Ranson's pyridine silver technic (McClung's Microscopical Technique, 2nd ed., p. 349) thru the dehydration step.

1. Double embedding: Absolute alcohol 2½ hr., change once; ether-alcohol, 2 hr.; 3½% celloidin, 2–4 days; 7% celloidin, 7–10 days or longer. Place tissue in paper box with 7% celloidin and harden in chloroform vapor 4–6 hr. Trim celloidin on four sides to within 1 mm. of tissue. Roughen two opposite sides of the block. Chloroform, 12–20 hr. Equal parts of chloroform and paraffin (m. p. 52° C.), 24 hr. at 55° C. Paraffin, 12 hr. at 55° C. Paraffin (m. p. 56–58° C.), 6–8 hr. Embed in the same. Mount and trim block so the roughened surfaces of celloidin block are at top and bottom. Cut sections 5 μ thick.

The following method is advised for attaching sections to the slide: Take one end of a strip of lens paper 2 cm. wide and long enough to encircle the slide and attach it to the under surface of the slide with 2% celloidin. Put a few sections on the upper surface of the slide and pull the lens paper tightly around the slide

over the sections and fix the end to the under surface of the slide with celloidin. Keep the slide horizontal while passing thru solutions.

2. Toning in $AuCl_3$: Chloroform, 2 min. to remove paraffin; 95% alcohol, 2 min.; other graded alcohols at intervals of 1 min. down to 50%; dist. water, 1 min.; 0.33% $AuCl_3$ to which has been added 10 drops of glacial acetic acid for each 50 cc. of solution, 30–50 sec. Remove quickly after sections have changed color. Rinse quickly in dist. water; 5% $Na_2S_2O_3$, 5 min.; tap water, 5 min.; dist. water, 1 min.; dehydrate by graded alcohols, at intervals of 1 min. up to 95% alcohol. If counterstain is not desired, dip for a few seconds in abs. alcohol; clear in oil of thyme and clove oil, 5:1. Mount as described later.

3. Counterstaining with azocarmine: Tone in $AuCl_3$ and dehydrate as described above. Mordant in anilin alcohol (1 cc. anilin oil in 1000 cc. of 95% alcohol), 15 min.; azocarmine (Grübler's) 1 g.; dist. water, 100 cc.; glacial acetic acid, 1 cc., in closed dish at 56° C., 15 min. Rinse in dist. water, removing excess only. Pass quickly thru graded alcohols, differentiate in anilin alcohol until cytoplasm becomes pinkish; 1 min. is usually sufficient. Grossly, sections remain a deep red. Acetic alcohol (1 cc. glacial acetic acid in 1000 cc. 95% alcohol), 1 min. to wash out anilin alcohol; 5% aq. phosphotungstic acid, 15 min.; 30 min. in the following stock solution diluted with equal parts of water: anilin blue, W. S., 0.5 g.; orange G, 2.0 g.; glacial acetic acid, 5.0 cc.; dist. water, 100 cc., dissolved by heating till it steams for 7 min.; filter after cooling. Rinse in dist. water, pass quickly thru graded alcohols (4–5 sec. in each), differentiate in 95% alcohol. Watch sections and remove when the nerve, very dark at first, becomes moderately blue. Pass quickly thru abs. alcohol, clear in oil of thyme and clove oil, 5:1; mount as follows: Drain the excess of clearing fluid; remove lens paper; blot on towel, section side down; straighten and remove wrinkles with camel's hair brush wet in clearing fluid. A small amount of thick balsam is spread on a cover slip and the cover slip is placed over the sections. Put a square piece of glass on the cover slip and hold it in place with a strong spring paper clip. The square of glass may be cut from a slide and should be slightly smaller than the cover slip. Dry slides at 40–50° C. for 1 or 2 days.

Results: Celloidin, by preserving the orientation of fibers, helps in the identification of closely packed axones. Toning in $AuCl_3$ practically clears the background of Ag-stained sections, while the unmyelinated axones appear black. Azocarmine does not stain the axis cylinders, but myelin sheaths stain orange, the nuclei of Schwann cells, light red, and the background, light blue.—*James B. Rogers.*

KINOSITA, R. and DAIKOKU, K. **A new fixative for tissue glycogen and the features of liver glycogen when it is applied.** *Trans. Jap. Path. Soc.*, **26**, 127–30. 1936.

Experiments in search of a fixative suitable in staining methods for tissue glycogen show that 95% alcohol alone, as well as saturated solutions of KCl or $MgSO_4$ in 95%, 80% and 50% alcohol are all equally good. The fixing properties of 4% formaldehyde, saturated with KCl or $MgSO_4$ are as good as those of diluted alcohol containing these chemicals. These methods are an advantage because they permit use of paraffin or celloidin which give thinner sections. KCl gives different results from $MgSO_4$. With the former, the boundary of each liver cell is distinct. The glycogen granules are finely divided and stain more brightly. They are aggregated more densely near the cell membrane and around the small vacuoles while they are more or less evenly distributed thruout the protoplasm. With $MgSO_4$ on the other hand, the boundary of each liver cell is indistinct and the glycogen occurs in the form of larger, irregularly grouped granules which are closely aligned around the small vacuoles of the cell. Formaldehyde fixation gives finer granules.

In another modification, a stronger solution of formaldehyde is used. When fairly large portions of tissue are to be fixed it is best to use 40% formaldehyde containing isotonic KCl.—*Elizabeth Bachelis.*

KONEFF, A. A. **An iron-hematoxylin-anilin-blue staining method for routine laboratory use.** *Anat. Rec.*, **66**, 173–9. 1936.

This method stains simultaneously most histological elements in paraffin, celloidin, or frozen sections after common fixatives, including Zenker and Zenker-for-

mol (which gives best results). Mordant sections in 5% aq. solution of $Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 + 24\ H_2O$ for 5–10 min. Rinse quickly in dist. water. Stain in Harris' hematoxylin for 3–15 min. (Good results have also been obtained with 0.5 to 1.0% alc. hematoxylin, freshly prepared or ripened; with 1% aq. hematoxylin prepared by boiling for 5–10 min.; and with Delafield's hematoxylin. The staining time must be determined for each type of hematoxylin.) Wash in dist. water. Place in the following mixture, which keeps well: anilin blue (Grübler), 0.1 g.; oxalic acid, 2.0 g.; phosphomolybdic acid, 15.0 g.; dist. water, 300 cc. Wash in dist. water, differentiate and dehydrate in 2 changes of abs. alcohol, clear in xylene, and mount in balsam. If euparal is used as the mounting medium, the xylene may be omitted. When staining loose celloidin sections, substitute 95% alc. for abs. alcohol and mount in euparal. Clearing in creosote is not necessary unless the sections are mounted in balsam.

The staining results are: nuclei, violet brown; cytoplasm, light brown; erythrocytes, dark violet; myelin and muscle fibers, brown; elastic fibers, reddish brown or red. This stain can be used in connection with reticulum impregnation and gives very sharp outlines.—*S. I. Kornhauser.*

LINDAHL, E. **Methode de décalcification des objects riches en carbonate de calcium.** *Bull. d'Histol. Appl.*, **12**, 216–9. 1935.

The author decalcifies delicate zoological objects (sponges, echinoderms, etc.) by converting their $CaCO_3$ into $Ca(HCO_3)_2$ (water soluble) by passing CO_2 thru an aqueous suspension either at atmospheric pressure or under a pressure of 3–4 atmospheres. A special apparatus is described. (*Cited from* Ber. wiss. Biol., **34**, 259. 1935.)—*J. M. Thuringer.*

MALLORY, F. B. **A lead hematoxylin stain for axis cylinders.** *Amer. J. Path.*, **12**, 569–71. 1936.

Fix 3 mm. tissue sections in 10% neutral formalin (time not specified). Mordant 6 weeks at room temp. or 7 days at 37° C. in sat. aq. solution of $PbCl_2$. Change fluid at end of 24 hr. and once or twice later. Wash 24 hr. in running water. Preserve in 80% alcohol. Embed in celloidin or paraffin. *Staining solution:* Dissolve 1–5 mg. hematoxylin in 1 cc. 95% alcohol and add 10 cc. sat. solution of $CaCO_3$ in dist. water. Coarse nerve fibers stain in 30–60 min. at room temp., fine fibers 3 hr. at 54° C. For a more differential stain, put unstained sections 1 min. into a solution of: I_2, 0.1 g.; KI, 0.2 g.; dist. water, 100 cc. Wash in water and 2 or 3 changes of 95% alcohol to extract iodine (do not use $Na_2S_2O_3$). Wash in water and stain as above. Dehydrate, clear, and mount.—*H. A. Davenport.*

MARTIN, J. F. and DELAUNAY, A. **Quelques points de technique pour les preparations histologiques du tissu osseux.** *Bull. d'Histol. Appl.*, **13**, 457–9. 1936.

Several suggestions are offered for improving the histological treatment of bony tissues. By holding a piece of bone gently between two cork discs in a vice, cut plates 1–2 cm. thick with a surgical saw. Fix (at least 2 weeks) in saline-formol, Bouin's, Helly's, or sublimate-formol-acetic liquid. Where acetic acid is specified the same volume of 5% trichloracetic acid will give less hemolysis. Fix 24–48 hr. in a vacuum as high as 70 mm. Wash at least 24 hr. in running water. Decalcify with 1% trichloracetic acid in the same degree of vacuum, changing once a day for about 8 days. Wash 24 hr. in running water until neutral to litmus. Dehydrate 48 hr. in abs. alcohol, changing twice. Embed by either of the two alternate procedures. (1) Soak 12 hr. in each of the following: 1:1 amyl acetate and abs. alcohol; amyl acetate; 1:1 amyl acetate and paraffin (52–54° C.); 3 changes of paraffin at 57° C. (2) After dehydration transfer to: methyl benzoate, 500 g.; celloidin, 5 g. In about 4 hr. the specimen will sink to the bottom. During the following 24 hr. change 3 times in the same liquid. Soak 24 hr. in benzine, changing once; transfer to 1:1 benzine and paraffin in an oven at 40° C. Follow 5 hr. in paraffin at 57° C., changing twice. Cool and block.

This procedure gives usual bone staining and preserves even the cytological details of the medullary tissue.—*J. A. de Tomasi.*

PRENANT, M. **Emploi du nitrate d'argent pour l'etude de la texture osseuse.** *Compt. Rend. Soc. Biol.*, **123**, 472–3. 1936.

Two modifications of the von Kossa silver method are proposed for better demonstration of the fine structure of compact bone: (1) Preliminary soaking of the bone in 0.04–0.09% agar to make diffusion of $AgNO_3$, uniform. (2) Impregnation with lower concentrations of $AgNO_3$ (0.5–0.005%).—*H. E. Jordan.*

SCHABADASCH, A. **Histophysiologie des réactions réciproques entre le bleu de méthylène et le tissu nerveux. III. Influence des ions sur la coloration. Signification particulière des ions-Mg. Conclusions générales.** *Bull. d'Histol. Appl.*, **13**, 137–51. 1936.

The conditions regulating vital staining of nervous tissue by methylene blue are discussed. The Mg ions in the following solution have a favorable effect: NaCl, 8 g.; glucose, 2 g.; sodium pyruvate, 0.324 g.; $MgBr_2$, 1.5 g.; methylene blue, 0.25 g.; dist. water, 1000 cc. Adjust to pH 6 with phosphate buffer. Injection of 1500 cc. into a cat gives a satisfactory specific staining of the peripheral and cerebrospinal nervous systems.

Conclusions: Methylene blue is an energetic receptor of H+. The presence of H ions causes substantial changes within the tissues and represents a necessary step toward the final reaction between the dye and the colloidal structures of nervous tissue. The presence of glucose or certain products of its metabolism (e. g., pyruvate) is also necessary. The addition to the dye of H receptors of the quinone group (resorcin and pyrocatechin) provides new methods for staining nervous tissue.—*J. A. de Tomasi.*

SEKI, M. **Zur Kenntnis der intra- und supravitalen Färbung. VII. Färbbarkeit der Blutmonocyten und ihre elektrische Ladung.** *Zts. Zellforsch. Mikr. Anat.*, **22**, 707–19. 1935.

The author concerns himself with the question of acidophilia and basophilia of the monocytes in comparison with other blood cells in various mammals. Stains employed: Pappenheim's panoptic stain, eosinate of methylene blue after methyl alcohol fixation, and methyl alcohol fixation followed by treatment with picric acid. All staining solutions were buffered to pH 7.0. The basophilia of the intergranular plasma substance measured by the intensity of the stain ranged from lymphocytes, monocytes, eosinophiles to special leukocytes. After treatment with picric acid which precipitated basic substances and removed the acid substances, it was found that the following serial arrangement indicated the acidophilic character of the plasma substance: monocytes, lymphocytes, special leukocytes, and eosinophiles. No appreciable differences in staining were noticed regardless of the animal from which the blood was obtained. The monocytes are very rich in acidophilic as well as basophilic substances, hence of a pronounced amphoteric or polyphase character. The acidophilic constituents of the cytoplasm colloids appear to play a significant role in vital staining with collargol, India ink and trypan blue. (*Cited from* Ber. wiss. Biol., **34**, 516. 1935.)—*J. M. Thuringer.*

STEWART, R. J. C. **A short Marchi technique.** *J. Path. & Bact.*, **43**, 339–43. 1936.

The Marchi technic, for degeneration of myelinated fibers, is open to several objections: Müller fixed material cannot be used with all stains; long storage in the fixative prevents satisfactory staining of the degenerated myelin; it is a lengthy procedure; and artefacts are fairly common. The following shortened Marchi schedule requires only 2 days (exclusive of fixation), overcomes most disadvantages of the original technic, and has been found satisfactory in over 3 years of routine application:

Fix pieces 2–3 mm. thick at least 2 days (preferably 8–10) in 10% neutralized formol-saline. Change after first 24 hr. Cut 30 μ frozen sections. Wash 1½ hr. in many changes of water, using dist. water for the last 4 washings. Place in 2.5% $K_2Cr_2O_7$ for 24 hr. at 21° C. Wash in dish of dist. water until *almost* all the yellow color disappears. Transfer to 1% osmic acid in the dark for 16–36 hr. The degree of blackening can be judged only by experience. The following treatment may be used if normal myelin is too dark. Wash 5 min. in tap water; treat 30 sec. with 0.05% $KMnO_4$; rinse with tap water; and hold in Pal's solution

(1% oxalic acid and 1% K_2SO_3, 1:1) until all brown deposit is removed. Wash in many changes of tap water. Pass thru 70% alcohol, 1–2 min.; 90% alcohol, 1–2 min.; abs. alcohol, 1–2 min. Mount in Gurr's neutral medium; harden 24 hr.; remove excess medium and ring with Krönig's Deckglaskitt (add gradually 8 parts colophonium resin to 2 parts melted paraffin wax, m. p. 54° C., mix well, cool).

The products of degenerated myelin are stained black. Alternative methods employing Scharlach R and methylene blue give less satisfactory results.—*J. A. de Tomasi.*

SZANTROCH, Z. **Beitrag zur Fettfärbung in den Gewebekulturen (Deckgläschenkulturen) and histologischen Schnitten mit Sudanlösung in Alkoholformol.** *Arch. exp. Zellforschung*, **17**, 206–9. 1935.

For simultaneous fixation and staining the author formerly proceeded as follows: Sat. solution of Sudan (*no further information available*) in 95% alcohol, 4 parts; 40% formalin, 1 part. Mix just before using. Stain from ½–5 min. Disadvantages of above method are: poor keeping qualities of preparations; confluence of fat droplets; and precipitates in culture media. The author now suggests the following improved method: 95% alcohol, 90 cc.; 40% formalin, 30 cc.; glacial acetic acid, 2 cc.; Sudan in excess. Let stand at room temp. for at least 2 weeks. Float preparation on stain from ½–5 min. Wash carefully. Counterstain in Delafield's hematoxylin. Mount in glycerin, seal cover glass with wax. For frozen sections, fix 24 hr. in 5% $K_2Cr_2O_7$, 8 parts; 40% formalin, 2 parts. Wash in running water for 24 hr. Stain 5–15 sec. (*Cited from* Ber. wiss. Biol., **34**. 1935.)—*J. M. Thuringer.*

PLANT MICROTECHNIC

CARLSON, J. G. **Effects of several fixatives on staining reactions in Zea mays, especially with reference to the Feulgen reaction.** *Cytologia*, **7**, 104–17. 1936.

The Feulgen stain is specific for chromatin, while hematoxylin can be made specific for any of the cell parts, depending on the fixative employed. This study is concerned with the effects of fixatives on staining. Root tips of *Zea mays* are fixed 40–48 hr. in the following fixatives: Bouin's, Navashin's, $CuCr_2O_7$, copper-chrome-propionate, nickel-chrome-propionate, formol-sulfuric acid, formic acid-acetaldehyde, modified Erlicki, and chromic sulfate-formaldehyde. Tissues are then washed 1 hr. in tap water, dehydrated, cleared, infiltrated and embedded by the butyl alcohol method. Slides are divided in two series, one of which is hydrolized for 20 min. at 60° C. in *N* HCl. Sections are stained with the Feulgen stain, Heidenhain's iron-alum-hematoxylin, crystal violet iodine, and safranin iodine (2% for 10 min.).

Results: The Feulgen reaction is chromatin-specific. The cuticle is also stained, showing a definite fixation effect because the color intensity varies with the nature of the fixative while specificity depended upon the type of chromatin stain. Fixation influences the staining reactions of different cell structures produced by hematoxylin, crystal violet and safranin. Crystal violet and safranin show consistent uniform selectivity. The HCl treatment affects the relative staining capacity of plastin and chromatin by hematoxylin, crystal violet and safranin, after some of the fixatives. HCl treatment after Bouin's and Navashin's fixatives lowers the stainability of chromatin by hematoxylin but improves the staining of nucleoli after Navashin's fixative. The effect of the HCl treatment upon the staining of chromatin or nucleoli by crystal violet and safranin varies definitely with the kind of fixative used.—*J. A. de Tomasi.*

HUSKINS, C. L. **On the cytology of speltoid wheats in relation to their origin and genetic behavior.** *J. Genetics*, **20**, 103–22. 1928.

Preliminary cytological fixations were made from greenhouse plants with Allen's Bouin, Carnoy (6:3:1 mixture), Flemming, and Kihara (Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B, **1**, 1–200. 1924) fixatives, and various modifications of the latter. Allen's Bouin gave the most uniform results, and a high general standard of fixation. Kihara's method, while somewhat variable, gave results

which were in most cases superior to any of the others. This method consists in fixing for 1 or 2 min. in Carnoy and then for 24 hr. in Flemming. In a modification of it which gave similar results, Zenker fluid with only 1 or 2% acetic acid was substituted for Flemming. This obviated the necessity of bleaching. Fixations of field-grown material were made principally by the Carnoy-Flemming or Carnoy-Zenker method. The anthers were dissected out in some cases, but fixations were chiefly made of spikelets with the ends of the glumes clipped off.

Newton's iodine-gentian-violet staining method (C. L. Huskins, J. Genetics, **18**, 315–64. 1927) was used almost exclusively, and gave results far superior to any other. It gave a particularly brilliant stain after the Carnoy-Zenker fixative.

All material was embedded in paraffin and cut, usually at 14 μ. The study was almost entirely confined to the pollen mother-cells, but a few examinations have been made of embryo-sac mother-cells and of root tips, the latter fixed in medium Flemming and Benda.—*R. W. Cumley.*

LUYET, B. J. **Differential staining for living and dead cells.** *Science*, **85**, 106. 1937.

Good results on plant material are secured with the following procedure: Lay on a slide, cutin side down, a portion of the lower epidermis of onion bulb. Cover 2 min. with a drop of 0.5% slightly alkalin neutral red solution. Blot and replace by 1 drop of 0.4% KOH. Blot immediately and wash with tap water. Living cells take a bright cherry red, dead cells are intense orange yellow. KOH is injurious in concentrations above 1%, but is not instantly lethal up to 2.5%.—*J. A. de Tomasi.*

MILOVIDOV, P. F. **Zur Theorie und Technik der Nuklealfärbung.** Protoplasma, **25**, 570–97. 1936.

The claims of several investigators that Feulgen's reaction does not occur in the cell nuclei of many plants are denied by the author who interprets the negative finds of others as the result of incomplete technic and, in many cases, may be due to the presence of tannates in the cell.—*R. Chambers.*

OURA, G. **A new method of unravelling the chromonema spirals.** *Zts. wiss. Mikr.*, **53**, 36–37. 1936.

Meiotic chromosomes in pollen mother-cells of *Tradescantia reflexa* are ordered in double coiled chromonema spirals. They can be unravelled by the following procedure. Spread out the cells on a slide, and add some aq. KCN (2^{-9} to 2^{-4} mol.) for a few minutes. Blot dry and stain with aceto-carmine. Heat the slide over the flame of a match. Other weak alkaline salts that can be used are NaOCl and $NaHCO_3$.—*J. A. de Tomasi.*

PHILP, J. and HUSKINS, C. L. **The cytology of Matthiola incana R. Br. especially in relation to the inheritance of double flowers.** *J. Genetics*, **24**, 359–404. 1931.

Root tips were taken from the seedlings and fixed in most of the standard fixatives used for plant cytology. Benda's fluid gave the best results. Ovules could not be fixed satisfactorily in any of the standard fluids tried, but good results were obtained from La Cour's first fixative (Nature, **124**, 127. 1929). Pollen mother-cell smears made according to Taylor's method (Bot. Gaz., **78**, 236–8. 1924) were tried, and a number of fixatives, including La Cour's and modifications of it, again gave the most satisfactory results. Satisfactory fixations of pollen mother-cells were also obtained by aceto-carmine methods. In making permanent aceto-carmine mounts prior fixation in acetic alcohol proved undesirable.—*R. W. Cumley.*

MICROÖRGANISMS

CHAPMAN, G. H. **Specificity of the dye in the crystal violet agar reaction of staphylococci.** *J. Bact.*, **32**, 199–205. 1936.

Staphylococci produce orange, violet or white growths on crystal violet agar. An attempt is made to find some explanation for these variable reactions, and to identify the active molecule of the dye. In order to determine the active chemical group, 10 strains of staphylococci are tested against 108 dyes, covering all the

range of biological dyes. Dilutions, in the agar base, of 1:10,000, 1:100,000, 1:250,000 and 1:500,000 are compared, in terms of growth color, with crystal violet (dilution 1:300,000 to 1:350,000). Significant color changes are only noted in the case of 5 samples of methyl violet, 1 of crystal violet, and 1 of Hofmann violet. Comparable results with brom thymol blue are attributed to its indicator properties.

A closer study of the violet phenylmethanes covers 60 samples including those labeled dahlia, dahlia B, crystal violet, methyl violet, gentian violet, ethyl violet, Hofmann violet. Samples of methylene violet were tested also. It appears that the only dyes capable of giving the color reaction are those suspected of containing methylated pararosanilins, particularly the higher homologs (penta- or hexamethyl pararosanilin). For this reason and because of its uniformity, Commission certified crystal violet is to be preferred for the preparation of crystal violet agar. Solutions of the dye may lose specificity on standing.—*J. A. de Tomasi.*

DIENST, R. B., and SANDERSON, E. S. **Use of nigrosin to demonstrate Treponema pallidum in syphilitic lesions.** *Amer. J. Pub. Health,* **26,** 910. 1936.

The characteristic morphology of this organism can best be demonstrated by darkfield illumination or by methods which stain the background, leaving the organism colorless. For the latter purpose the authors find nigrosin far superior to other agents. Successful results depend upon the selection of the most suitable material for examination and the most suitable method of collection. Exudate should be obtained by squeezing cleaned and dried primary ulcers or papules. Glandular fluid should be obtained with a sterile syringe.

Wet preparation: Place a small loopful of the exudate on a clean slide and add a loopful of 5% aq. nigrosin to which 0.5% formalin has been added. Mix thoroly and spread over slide so that part of smear is thin. Pass slide over flame to dry rapidly. Place drop of immersion oil directly on smear and examine.

Dry preparation: Allow a drop of exudate to air dry on a slide. This may be sent to the laboratory and stained even as much as 45 days later, altho the sooner the better. To stain, place a small loopful of water on the dried exudate, allow to diffuse for a few seconds, then apply a loopful of the nigrosin solution, smear, dry, and examine as before.

This method checked well with Wassermann, Kahn, and darkfield reactions, and is proposed to replace darkfield examinations in state laboratories.—*M. W. Jennison.*

GELARIE, A. J. **A new, one-minute method for the staining of spirochetes, spirilla, spermatozoa, and related organisms.** *J. Lab. & Clin. Med.,* **21,** 1065–9. 1936.

Shortcomings of Burri's, Levaditi's, and Giemsa's methods for the examination of fluids containing spirochetes and spirilla are pointed out. Directions for a quick method are: Smear the material thinly and air-dry. Fix 5 sec. in a zirconyl chloride solution (2.5 g. recrystallized $ZrOCl_2$ dissolved in 100 cc. 10% aq. NaCl). Wash with water. Treat 10 sec. with a few drops of 10% aq. recrystallized citric acid solution (preserve with thymol). Wash with water. Stain 30 sec. with 0.25% gentian violet in $N/10$ sodium lactate (approx. pH 8.4). Wash with water. Cover a few seconds with K_2HgI_4 solution (dissolve 0.5 g. HgI_2 in 100 cc. 0.5% aq. KI). Wash with water. Counterstain 5 sec. with carbol methylene blue (dissolve 0.2 g. methylene blue in 100 cc. dist. water, add 0.2 g. phenol). Wash and blot dry. Red blood cells show finely granular rose colored cytoplasm; chromatin of leucocytes, blue; spirilla, deep purple. Mechanism of the stain is explained on a chemico-physical basis, assuming differences in the isoelectric points of constituents of organisms and those of red blood cells. Substitution of Al, Bi, Cd, Mg, Hg, Th, U, Cu and Sn chlorides does not give satisfactory results.—*J. A. de Tomasi.*

MALAKOFF, M. T. **A technique for the slide culture of fungi.** *Science,* **84,** 490. 1936.

A method is given for setting up slide cultures of various fungi which are also suitable for permanent mounting. Sterilize the slide in individual 30 x 140 mm. test tubes. Melt sterile agar medium, tubed in 3–5 cc. quantities, and cool to

point of gelation. Pour aseptically over the slide. This affords hardening of medium in an uneven layer which upon inoculation permits all stages of growth. In 48 hr. at room temperature slides are dry enough for mounting. Fix in: 50% alcohol, 100 cc.; formalin, 6.5 cc.; glacial acetic acid, 2.5 cc. Pass thru 35% alcohol and dist. water. Stain 5 min. in an acid dye, wash thoroly in dist. water, dry overnight and mount.—*J. A. de Tomasi.*

TSUCHIYA, H. **The effects of dyes on Endameba histolytica in vitro.** *J. Lab. & Clin. Med.*, **21**, 1028–35. 1936.

A study is reported of the viability of cysts and trophozoites of *E. histolytica* when exposed to the action of various dyes, with a view to determining any changes of cell structure or any dye resistance developed. For the viability of cysts, eosin proves to be quite inadequate. Other dyes used are: gentian violet, malachite green, brilliant green, pyoktanin, acid and basic fuchsin, acriflavine, neutral red, methylene blue, safranin, brilliant cresol blue, toluidine blue, Bismarck brown, eosin B and mercurochrome. To 5 cc. of dye dilution, 0.1 cc. of washed cysts is added. After incubation at various temperatures and at varying time intervals, 0.1 cc. is diluted in 0.9 cc. nutrient broth, and 0.1 cc. of the dilution is inoculated into the author's S. C. culture medium with Dorsett's egg slant (Tsuchiya, H. Further studies on the cultivation of *Endameba histolytica* and a complement fixation test for amebiasas. J. Lab. & Clin. Med., **19**, 495. 1934).

Upon incubation at 37° C. the culture is examined in fresh preparation, or stained with iron hematoxylin, Tsuchiya's technic (Tsuchiya, H. A practical staining method for intestinal protozoa. J. Lab. & Clin. Med., **17**, 1163. 1932) or Gram's technic. To study the effect of dyes on the trophozoites, 0.5 cc. of each dilution of dye is added to 4.5 cc. of a growing culture, taking observations at 24, 48, and 72 hr. at 37° C. Surviving cultures are transferred to fresh culture medium and exposed to equal dye concentrations. High concentrations of the dye have an amebicidal action on cysts. Dilution results in amebastasis, displayed best by gentian violet, acriflavine, malachite green, and to a lesser extent by basic and acid fuchsins. Amebastasis is accompanied by an increased size of the organisms. Action on trophozoites is usually amebicidal. Resistance to gentian violet, acid fuchsin and malachite green can be built up.—*J. A. de Tomasi.*

HISTOCHEMISTRY

FAUTREZ, J. **Sur le point isoélectrique des cellules du système réticulo-endothélial des vertébrés.** *Bull. d'Histol. Appl.*, **13**, 202–6. 1936.

The isoelectric point of the cytoplasm of reticulo-endothelial cells is found to lie in the same pH region as in the case of other cells. This point has been investigated by staining sections of the tissue 10 min. with cyanol, an acid dye, and toluidine blue, a basic dye, at various pH. Results do not agree with Seki's findings (Seki, M. Zts. Zellforsch., **19**, 238. 1933).—*J. A. de Tomasi.*

GÖMÖRI, G. **Microtechnical demonstration of iron.** *Amer. J. Path.*, **12**, 655–63. 1936.

Formalin and formalin-alcohol (no formulae specified) fixed organs containing iron bearing pigments were subjected to standard staining methods for iron. No traces of ferrous compounds were found by the direct Turnbull's blue reaction. The best microtechnical reagent for Fe was found to be a mixture of equal parts of 1:4 HCl and 10% $K_4Fe(CN)_6$. Sections should be soaked 30 min. Results of similar quality were obtained by treating with $(NH_4)_2S$ (Quincke's reagent) 1 hr., washing with dilute NH_4OH, dist. water, and finally 1 hr. in 5% $CuSO_4$ or $Pb(NO_3)_2$. Fading of Berlin blue and Turnbull's blue preparations was prevented by thinning nearly dry Canada balsam with oxidized oil of turpentine.—*H. A. Davenport.*

LUCAS, M. S. and EVANS, C. A. **Correlation of qualitative microchemical tests on the protozoan nucleus and the mode of nutrition.** *J. Royal Micros. Soc.*, **55**, 261–4. 1935.

This paper is a discussion of the relationship between the chromatin of the nucleus in certain protozoa, as revealed by the Feulgen technic, and the mineral

constituents of it, as shown by microincineration. The following observations are made: the protozoan nucleus does not necessarily contain its nucleic acid in the form of thymonucleic acid. There are instances where chromatin in the fixed nucleus stains with basophilic dyes, but reacts negatively with the Feulgen test. This cannot, therefore, be considered a universal test for chromatin. Furthermore, the nucleus in saprozoic protozoa is deficient in inorganic salts which will withstand incineration. These two main points cast doubt on the reliability of comparisons between protozoan and metazoan nuclei.—*J. A. de Tomasi.*

MARZA, V. D. and CHIOSA, L. T. **Histochimie quantitative du potassium dans les ovules en croissance.** *Bull. d'Histol. Appl.*, **13**, 153–77. 1936.

The role of K in biological processes is known to be a very important one. This extensive paper deals with the part played by this element in cellular metabolism as determined by studying the quantitative histochemical variations in hen's ova. Twelve ovaries are fixed in 96% alcohol, dehydrated in abs. alcohol, cleared, embedded in paraffin and cut 7 μ thick. Determination of K is carried out by means of the microcomparator (Marza, V. D. and Chiosa, L. Essai de detection histochimique quantitative du potassium. Bull. d'histol. Appl., **12**, 58–72. 1935).

Results: K displays slight tendency to concentrate in ovular membranes. Its accumulation in the ovum is in direct relation to the appearance of the vitellus.—*J. A. de Tomasi.*

URAKAMI, T. **Ein neues Verständniss des Unnaschen Sauerstoffortes und Reduktionsortes.** *Trans. Soc. Path. Jap.*, **24**, 96–103. 1934.

Histological staining is based upon an adsorption phenomenon between 2 colloids having opposite charges, i. e., the tissue protein and the dye. The least staining, therefore, occurs at the iso-electric point of the tissue protein and conversely the disappearance of the staining may be used as an indicator of the i. e. p. at a given pH.

Technic: Fresh formalin, or alcohol fixed sections are stained with methylene blue or crystal ponceau solutions, buffered in steps from pH 2 to 7 and washed with corresponding buffer solutions, treated with 4% $(NH_4)_2MoO_4$, alcohol, xylene, balsam. The results are presented in a table. Ordinarily the i. e. p. of the nucleus is more on the acid side than the cytoplasm. Hardening with formalin produces a shift of the i. e. p. toward the acid side, with alcohol toward the alkaline side. The author rejects Unna's theory of oxidation and reduction regions in cells, assuming that the differences in staining with the rongalit white technic were not brought about thru variations in oxidation or reduction intensity but thru differences in pH which changed the electrical charge and with it the staining qualities. (*Cited from* Ber. wiss. Biol., **34**, 516. 1935.)—*J. M. Thuringer.*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stains approved since the last one listed in the January number of this Journal.

STAINS CERTIFIED DECEMBER 1, 1936 TO FEBRUARY 28, 1937*

Name of dye	Certification No. of batch	Dye Content	Object of tests made by commission†	Date approved
Alizarin red S	NAr 1	——	For staining bone	Dec. 4, 1936
Alizarin red S	LAr 1	——	For staining bone	Dec. 4, 1936
Crystal violet	CC 8	88%	As histological, cytological and bacteriological stain	Dec. 19, 1936
Fast green FCF	NGf 3	93%	As histological counterstain	Jan. 4, 1937
Pyronin G	NP 6	58%	As constituent of Pappenheim stain	Jan. 12, 1937
Tetrachrome stain	NMn 9	——	As blood stain	Jan. 23, 1937
Cresyl violet	NW 6	92%	For use in histology	Feb. 25, 1937

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.